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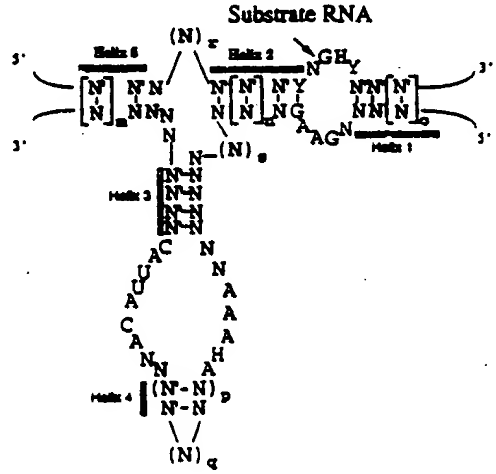
(54) Title: **METHOD AND REAGENT FOR INHIBITING THE EXPRESSION OF DISEASE RELATED GENES**

Substrate RNA



(57) Abstract

Enzymatic RNA molecules which cleave ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF-α mRNA, RSV mRNA or RSV genomic RNA, or CML associated mRNA, and use of these molecules for the treatment of pathological conditions related to those mRNA-levels; ribonucleosides or nucleotides modified in 2', 3' or 5', methods for their synthesis, purification and deprotection; vectors containing multiple enzymatic nucleic acids, optionally in chimeric form with tRNAs; method for introducing enzymatic nucleic acids into cells by forming a complex with a second nucleic acid, where the complex is capable of taking an R-loop base-paired structure; method for altering a mutant nucleic acid *in vivo* by hybridization with an oligonucleotide capable of activating dsRNA deaminase, comprising an enzymatic activity or a chemical mutagen. Further are disclosed trans-cleaving or -ligating hairpin ribozymes lacking a substrate RNA moiety, as well as hammerhead ribozymes having an interconnecting loop between base pairs in stem II.

METHOD AND REAGENT FOR INHIBITING THE EXPRESSION OF DISEASE RELATED GENES

Background of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as inflammatory or autoimmune disorders, chronic myelogenous leukemia, or respiratory tract illness.

Summary of the Invention

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting the expression of disease related genes, e.g., ICAM-1, IL-5, relA, TNF-α, p210 $^{bcr-abl}$, and respiratory syncytial virus genes. Such ribozymes can be used in a method for treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be targeted to virtually any RNA transcript, and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table 1 summarizes some of the characteristics of these ribozymes.

Ribozymes act by first binding to a target RNA. Such binding occurs through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA which acts to cleave the target RNA. Thus, the ribozyme first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After a ribozyme has bound and cleaved its RNA target it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the effective concentration of ribozyme necessary to effect a therapeutic treatment is lower than that of an antisense oligonucleotide. The advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ration of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, it is thought that the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site. With their catalytic activity and increased site specificity, ribozymes represent more potent and safe therapeutic molecules than antisense oligonucleotides.

Thus, in a first aspect, this invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave RNA species encoding ICAM-1, IL-5, relA, TNF-α, p210$^{bcr\text{-}abl}$, or RSV proteins. In particular, applicant describes the selection and function of ribozymes capable of cleaving these RNAs and their use to reduce levels of ICAM-1, IL-5, relA, TNF-α, p210 bor-abl or RSV proteins in various tissues to treat the diseases discussed herein. Such ribozymes are also useful for diagnostic uses.

Applicant indicates that these ribozymes are able to inhibit expression of ICAM-1, IL-5, rel A, TNF-α, p210$^{bcr\text{-}abl}$, or RSV genes and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave target ICAM-1, IL-5, rel A, TNF-α, p210$^{bcr\text{-}abl}$, or RSV encoding mRNAs may be readily designed and are within the invention.

These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the

cleavage of RNA. Upon binding, the ribozymes cl ave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleaic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for examplke, Hoogsteen type) of base-paired interactions.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in associateion with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses* , 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel et al., 1990, *Nucleic Acids Res.* 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 16 of the RNaseP motif by Guerrier-Takada et al., 1983 *Cell*, 35 849,

Neurospora VS RNA ribozyme motif is described by Collins (S ville and Collins, 1990 *Cell* 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci., USA 88, 8826-8830; Collins and Olive, 1993 *Biochemistry* 32, 2795-2799 Guo and Collins, 1995 EMBO. J., 14, 368) and of the Group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it has nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target (i.e., I CAM-1, IL-5, reLA, TNF-α, p210 $^{bcr-abl}$ or RSV proteins) encoding mRNA such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required., Alternatively, the ribozymes can be expressed from vectors that are delivered to specific cells. By "vectors" is meant any nucleic acid and/or viral-based technique used to deliver a desired nucleic acid.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structrure. However, these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g. Scanlon, K.J. et al., 1991, *Proc. Natl. Acad. Sci., USA*, 88, 10591-5; Kashani-Sabet, M., et al.,1992, *Antisense Res. Dev.*, 2, 3-15; Dropoulic, B., et al., 1992, *J. Virol*, 66, 1432-41; Weerasinghe, M., et al., 191, *J. Virol.* 65, 5531-4; Ojwang, J.O., et al., 1992, *Proc. Natl. Acad. Sci., USA*, 89 10802-6; Chen C.J., et al., 1992, Nucleic Acids R s., 20, 4581-9; Sarver, H., et al., 1990 *Science*, 247, 1222-1225). Those skilled in the art would realize that any ribozyme can be

expressed in eukaryotic cells from the appropriate DNA or RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa, J., et al., 1992, *Nucleic Acids Symp. Ser.* 27, 15-6; Taira, K. et al., *Nucleic Acids Res.*, 19, 5125-30; Ventura, M., et al., 1993, *Nucleic Acids Res.*, 21, 3249-55, Chowrira et al., 1994 *J. Biol. Chem.*, 269, 25856).

By "inhibit" is meant that the activity or level of ICAM-1,Rel A, IL-5, TNF-α, p210$^{bcr-abl}$ or RSV encoding mRNA is reduced below that observed in the absense of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of ICAM-1, IL-5, Rel A, TNF-α, p210$^{bcr-abl}$ or RSV protein or activity in a cell or tissue. By "related" is meant that the inhibition of ICAM-1, IL-5, Rel A, TNF-α, p210$^{bcr-abl}$ or RSV mRNA translation, and thus reduction in the level of, ICAM-1, IL-5, Rel A, TNF-α, p210$^{bcr-abl}$ or RSV proteins will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 2,3,6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36 and 37.

Examples of such ribozymes are shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequence listed in the above identified Tables may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit ICAM-1, IL-5, Rel A, TNF-α, $p210^{bcr-abl}$ or RSV gene expression are expressed from transcription units inserted into DNA, RNA, or viral vectors. Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. USA*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al., 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. USA*, 90, 6340-4; L'Huiller et al., 1992 *EMBO J.* 11, 4411-8; Lisziewicz et al., 1993 *Proc. Natl. Acad. Sci. U.S.A.*, 90 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2(a) is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2(b) is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure 2(c) is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2(d) is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1,2,3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3-20 bases, *i.e.*, m is from 1-20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4-20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each independently from 0 to any number, *e.g.* 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be

modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a diagrammatic representation of the genetic map of RSV strain A2.

Figure 7 is a diagrammatic representation of the solid-phase synthesis of RNA.

Figure 8 is a diagrammatic representation of exocyclic amino protecting groups for nucleic acid synthesis.

Figure 9 is a diagrammatic representation of the deprotection of RNA.

Figure 10 is a graphical representation of the cleavage of an RNA substrate by ribozymes synthesized, deprotected and purified using the improved methods described herein.

Figure 11 is a schematic representation of a two pot deprotection protocol. Base deprotection is carried out with aqueous methyl amine at 65 °C for 10 min. The sample is dried in a speed-vac for 2-24 hours depending on the scale of RNA synthesis. Silyl protecting group at the 2'-hydroxyl position is removed by treating the sample with 1.4 M anhydrous HF at 65°C for 1.5 hours.

Figure 12 is a schematic representation of a one pot deprotection of RNA synthesized using RNA phosphoramidite chemistry. Anhydrous methyl amine is used to deprotect bases at 65°C for 15 min. The sample is allowed to cool for 10 min before adding TEA•3HF reagent, to the same

pot, to remove protecting groups at the 2'-hydroxyl position. The deprotection is carried out for 1.5 hours.

Figs. 13a - b is a HPLC profile of a 36 nt long ribozyme, targeted to site B. The RNA is deprotected using either the two pot or the one pot deprotection protocol. The peaks corresponding to full-length RNA is indicated. The sequence for site B is CCUGGGCCAGGGAUUA AUGGAGAUGCCCACU.

Figure 14 is a graph comparing RNA cleavage activity of ribozymes deprotected by two pot vs one pot deprotection protocols.

Figure 15 is a schematic representation of an improved method of synthesizing RNA containing phosphorothioate linkages.

Figure 16 shows RNA cleavage reaction catalyzed by ribozymes containing phosphorothioate linkages. Hammerhead ribozyme targeted to site C is synthesized such that 4 nts at the 5' end contain phosphorothioate linkages. P=O refers to ribozyme without phosphorothioate linkages. P=S refers to ribozyme with phosphorothioate linkages. The sequence for site C is UCAUUUUGGCCAUCUC UUCCUUCAGGCGUGG.

Figure 17 is a schematic representation of synthesis of 2'-N-phtalimido-nucleoside phosphoramidite.

Figure 18 is a diagrammatic representation of a prior art method for the solid-phase synthesis of RNA using silyl ethers, and the method of this invention using SEM as a 2'-protecting group.

Figure 19 is a diagrammatic representation of the synthesis of 2'-SEM-protected nucleosides and phosphoramidites useful for the synthesis of RNA. B is any nucleotide base as exemplified in the Figure, P is purine and I is inosine. Standard abbreviations are used throughout this application, well known to those in the art.

Figure 20 is a diagrammatic representation of a prior art method for deprotection of RNA using TBDMS protection of the 2'-hydroxyl group.

Figure 21 is a diagrammatic representation of the deprotection of RNA having SEM protection of the 2'-hydroxyl group.

Figure 22 is a representation of an HPLC chromatogram of a fully deprotected 10-mer of uridylic acid.

Figs. 23 - 25 are diagrammatic representations of hammerhead, hairpin or hepatitis delta virus ribozyme containing self-processing RNA transcript. Solid arrows indicate self-processing sites. Boxes indicate the sites of nucleotide substitution. Solid lines are drawn to show the binding sites of primers used in a primer-extension assay. Lower case letters indicate vector sequence present in the RNA when transcribed from a *Hin*dIII-linearized plasmid. **(23)** HH Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hammerhead ribozyme. The structure of the hammerhead ribozyme is based on phylogenetic and mutational analysis (reviewed by Symons, 1992 supra). The trans ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (UC at positions 50 and 51) at its 3' end. The 3' processing ribozyme is comprised of nucleotides 44 through 96. Roman numerals I, II and III, indicate the three helices that contribute to the structure of the 3' cis-acting hammerhead ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20, 3252). Substitution of G_{70} and A_{71} to U and G respectively, inactivates the hammerhead ribozyme (Ruffner et al., 1990 Biochemistry 29, 10695) and generates the HH(mutant) construct. **(24)** HP Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hairpin ribozyme. The structure of the hairpin ribozyme is based on phylogenetic and mutational analysis (Berzal-Herranz et al., 1993 EMBO. J 12, 2567). The trans-ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 5 non-ribozyme nucleotides (UGGCA at positions 50 to 54) at its 3' end. The 3' cis-acting ribozyme is comprised of nucleotides 50 through 115. The transcript named HP(GU) was constructed with a potential wobble base pair between G_{52} and U_{77}; HP(GC) has a Watson-Crick base pair between G_{52} and C_{77}. A shortened helix 1 (5 base pairs) and a stable tetraloop (GAAA) at the end of helix 1 was used to connect the substrate with the catalytic domain of the hairpin ribozyme (Feldstein & Bruening, 1993 Nucleic Acids Res. 21, 1991; Altschuler et al., 1992 supra). **(25)** HDV Cassette, transcript containing the trans-acting hammerhead ribozyme linked to a 3' cis-acting hepatitis delta virus (HDV) ribozyme. The secondary structure of the HDV ribozyme is as proposed by Been and

coworkers (Been et al., 1992 Biochemistry 31, 11843). The trans-ribozyme domain ext nds from nucleotides 1 through 48. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (AA at positions 49 to 50) at its 3' end. The 3' cis-acting HDV ribozyme is comprised of nucleotides 50 through 114. Roman numerals I, II, III & IV, indicate the location of four helices within the 3' cis-acting HDV ribozyme (Perrota & Been, 1991 Nature 350, 434). The ΔHDV transcript contains a 31 nucleotide deletion in the HDV portion of the transcript (nucleotides 84 through 115 deleted).

Fig. 26 is a schematic representation of a plasmid containing the insert encoding self-processing cassette. The figure is not drawn to scale.

Fig. 27 demonstrates the effect of 3' flanking sequences on RNA self-processing *in vitro*. **H**, Plasmid templates linearized with *Hin*dIII restriction enzyme. Transcripts from **H** templates contain four non-ribozyme nucleotides at the 3' end. **N**, Plasmid templates linearized with *Nde*I restriction enzyme. Transcripts from **N** templates contain 220 non-ribozyme nucleotides at the 3' end. **R**, Plasmid templates linearized with *Rca*I restriction enzyme. Transcripts from **R** templates contain 450 non-ribozyme nucleotides at the 3' end.

Fig. 28 shows the effect of 3' flanking sequences on the trans-cleavage reaction catalyzed by a hammerhead ribozyme. A 622 nt internally-labeled RNA (<10 nM) was incubated with ribozyme (1000 nM) under single turn-over conditions (Herschlag and Cech, 1990 Biochemistry 29, 10159). **HH+2, HH+37**, and **HH+52** are trans-acting ribozymes produced by transcription from the HH, ΔHDV, and HH(mutant) constructs, respectively, and that contain 2, 37 and 52 extra nucleotides on the 3' end. The plot of the fraction of uncleaved substrate versus time was fit to a double exponential curve using the KaleidaGraph graphing program (Synergy Software, Reading, PA). A double exponential curve fit was used because the data points did not fall on a single exponential curve, presumably due to varying conformers of ribozyme and/or substrate RNA.

Fig. 29 shows RNA self-processing in OST7-1 cells. *In vitro* lanes contain full-length, unprocessed transcripts that were added to cellular lysates prior to RNA extraction. These RNAs were either pre-incubated with $MgCl_2$ (+) or with DEPC-treated water (–) prior to being hybridized

with 5' end-labeled primers. Cellular lanes contain total cellular RNA from cells transfected with one of the four self-processing constructs. Cellular RNA are probed for ribozyme expression using a sequ nce specific primer-extension assay. Solid arrows indicate the location of primer extension bands corresponding to Full-Length RNA and 3' Cleavage Products.

Figs. 30,31 are diagrammatic representations of self-processing cassettes that will release trans-acting ribozymes with defined, stable stem-loop structures at the 5' and the 3' end following self-processing. 30, shows various permutations of a hammerhead self-processing cassette. 31, shows various permutations of a hairpin self-processing cassette.

Figs. 32a-b Schematic representation of RNA polymerse III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figs. 33a-e Sequence of the primary $tRNA_i^{met}$ and Δ3-5 transcripts. The A and B box are internal promoter regions necessary for pol III transcription. Arrows indicate the sites of endogenous tRNA processing. The Δ3-5 transcript is a truncated version of tRNA wherein the sequence 3' of B box has been deleted (Adeniyi-Jones et al., 1984 *supra*). This modification renders the Δ 3-5 RNA resistant to endogenous tRNA processing.

Figure 34. Schematic representation of RNA structural motifs inserted into the Δ3-5 RNA. Δ3-5/HHI- a hammerhead (HHI) ribozyme was cloned at the 3' region of Δ3-5 RNA; S3- a stable stem-loop structure was incorporated at the 3' end of the Δ3-5/HHI chimera; S5- stable stem-loop structures were incorporated at the 5' and the 3' ends of Δ3-5/HHI ribozyme chimera; S35- sequence at the 3' end of the Δ3-5/HHI ribozyme chimera was altered to enable duplex formation between the 5' end and a complementary 3' r gion of th same RNA; S35Plus- in addition to structural alterations of S35, sequences were altered to facilitate additional

duplex formation within the non-ribozyme sequence of the Δ3-5/HHI chimera.

Figures 35 and 36. Northern analysis to quantitate ribozyme expression in T cell lines transduced with Δ3-5 vectors. 35) Δ3-5/HHI and its variants were cloned individually into the DC retroviral vector (Sullenger et al., 1990 *supra*). Northern analysis of ribozyme chimeras expressed in MT-2 cells was performed. Total RNA was isolated from cells (Chomczynski & Sacchi, 1987 *Analytical Biochemistry* 162, 156-159), and transduced with various constructs described in Fig. 34. Northern analysis was carried out using standard protocols (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Nomenclature is same as in Figure 34. This assay measures the level of expression from the type 2 pol III promoter. 36) Expression of S35 constructs in MT2 cells. S35 (+ribozyme), S35 construct containing HHI ribozyme. S35 (-ribozyme), S35 construct containing no ribozyme.

Figure 37. Ribozyme activity in total RNA extracted from transduced MT-2 cells. Total RNA was isolated from cells transduced with Δ3-5 constructs described in Figs. 35 and 36 In a standard ribozyme cleavage reaction, 5 µg total RNA and trace amounts of 5' terminus-labeled ribozyme target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris-HCl, pH 7.5 and 10 mM $MgCl_2$. RNAs were renatured by cooling the reaction mixture to 37°C for 10-15 min. Cleavage reaction was initiated by mixing the labeled substrate RNA and total cellular RNA at 37°C. The reaction was allowed to proceed for ~ 18h, following which the samples were resolved on a 20 % urea-polyacrylamide gel. Bands were visualized by autoradiography.

Figures 38 and 39. Ribozyme expression and activity levels in S35-transduced clonal CEM cell lines. 38) Northern analysis of S35-transduced clonal CEM cell lines. Standard curve was generated by spiking known concentrations of in vitro transcribed S5 RNA into total cellular RNA isolated from non-transduced CEM cells. Pool, contains RNA from pooled cells transduced with S35 construct. Pool (-G418 for 3 Mo), contains RNA from pooled cells that were initially selected for resistance to G418 and then grown in the absence of G418 for 3 months. Lanes A through N contain RNA from individual clones that were generated from the pooled cells transduced with S35 construct. $tRNA_i^{met}$, refers to the

endogenous tRNA. S35, r fers to the position of the ribozyme band. M, marker lane. 39) Activity levels in S35-transduced clonal CEM cell lines. RNA isolation and cleavage reactions were as described in Fig.37. Nomenclature is same as in Figs. 35 and 36 except, S, 5' terminus-labeled substrate RNA. P, 8 nt 5' terminus-labeled ribozyme-mediated RNA cleavage product.

Figures 40 and 41 are proposed secondary structures of S35 and S35 containing a desired RNA (HHI), respectively. The position of HHI ribozyme is indicated in figure 41. Intramolecular stem refers to the stem structure formed due to an intramolecular base-paired interaction between the 3' sequence and the complementary 5' terminus. The length of the stem ranges from 15-16 base-pairs. Location of the A and the B boxes are shown.

Figures 42 and 43 are proposed secondary structures of S35 plus and S35 plus containing HHI ribozyme.

Figures 44, 45, 46 and 47 are the nucleotide base sequences of S35, HHIS35, S35 Plus, and HHIS35 Plus respectively.

Figs. 48a-b is a general formula for pol III RNA of this invention.

Figure 49 is a digrammatic representation of 5T construct. In this construct the desired RNA is located 3' of the intramolecular stem.

Figures 50 and 51 contain proposed secondary structures of 5T construct alone and 5T contruct containing a desired RNA (HHI ribozyme) respectively.

Figure 52 is a diagrammatic representation of TRZ-tRNA chimeras. The site of desired RNA insertion is indicated.

Figure 53 shows the general structure of HHITRZ-A ribozyme chimera. A hammerhead ribozyme targeted to site I is inserted into the stem II region of TRZ-tRNA chimera.

Figure 54 shows the general structure of HPITRZ-A ribozyme chimera. A hairpin ribozym targ ted to site I is cloned into th indicated region of TRZ-tRNA chim ra.

Figure 55 shows a comparison of RNA cleavage activity of HHITRZ-A, HHITRZ-B and a chemically synthesized HHI hammerh ad ribozymes.

Figure 56 shows expression of ribozymes in T cell lines that are stably transduced with viral vectors. M, markers; lane 1, non-transduced CEM cells; lanes 2 and 3, MT2 and CEM cells transduced with retroviral vectors; lanes 4 and 5, MT2 and CEM cells transduced with AAV vectors.

Figs. 57a-b Schematic diagram of adeno-associated virus and adenovirues vectors for ribozyme delivery. Both vectors utilize one or more ribozyme encoding transcription units (RZ) based on RNA polymerase II or RNA polymerase III promoters. A. Diagram of an AAV-based vector containing minimal AAV sequences comprising the inverted terminal repeats (ITR) at each end of the vector genome, an optional selectable marker (Neo) driven by an exogenous promoter (Pro), a ribozyme transcription unit, and sufficient additional sequences (stuffer) to maintain a vector length suitable for efficient packaging. B. Diagram of ribozyme expressing adenovirus vectors containing deletions of one or more wild type adenoviorus coding regions (cross-hatched boxes marked as E1, pIX, E3, and E4), and insertion of the ribozyme transcription unit at any or several of those regions of deletions.

Fig. 58 is a graph showing the effect of arm length variation on the activity of ligated hammerhead (HH) ribozymes. Nomenclature 5/5, 6/6, 7/7, 8/8 and so on refers to the number of base-pairs being formed between the ribozyme and the target. For example, 5/8 means that the HH ribozyme forms 5 bp on the 5' side and 8 bp on the 3' side of the cleavage site for a total of 13 bp. $-\Delta G$ refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 Ann. Rev. Biophys. Chem. 17, 167). RPI A is a HH ribozyme with 6/6 binding arms.

Figs. 59 and 60 and 61 show cleavage of long substrate (622 nt) by ligated HH ribozymes.

Fig. 62 is a diagrammatic representation of a hammerhead ribozyme (HH-H) targeted against a site termed H. Variants of HH-H are also shown that contain either a 2 base-paired stem II (HH-H1 and HH-H2) or a 3 base-paired stem II (HH-H3 and HH-H4).

Figs. 63 and 64 show RNA cleavage activity of HH-I and its variants (see Fig.62). 63) cleavage of matched substrate RNA (15 nt). 64) cleavage of long substrate RNA (613 nt).

Figs. 65a-b is a schematic representation of a method of this invention to synthesize a full length hairpin ribozyme. No splint strand is required for ligation but rather the two fragments hybridize together at helix 4 prior to ligation. The only prerequisite is that the 3' fragment is phosphorylated at its 5' end and that the 3' end of the 5' fragment have a hydroxyl group. The hairpin ribozyme is targeted against site J. H1 and H2 are intermolecular helices formed between the ribozyme and the substrate. H3 and H4 are intramolecular helices formed within the hairpin ribozyme motif. Arrow indicates the cleavage site.

Fig. 66 shows RNA cleavage activity of ligated hairpin ribozymes targeted against site J.

Figs. 67a-b is a diagrammatic representation of a Site K Hairpin Ribozyme (HP-K) showing the proposed secondary structure of the hairpin ribozyme •substrate complex as described in the art (Berzal-Herranz *et al.*, 1993 *EMBO. J.*12, 2567). The ribozyme has been assembled from two fragments (bimolecular ribozyme; Chowrira and Burke, 1992 *Nucleic Acids Res.* 20, 2835); #H1 and H2 represent intermolecular helix formation between the ribozyme and the substrate. H3 and H4 represent intramolecular helix formation within the ribozyme (intermolecular helix in the case of bimolecular ribozyme). Left panel (HP-K1) indicates 4 base-paired helix 2 and the right panel (HP-K2) indicates 6 base-paired helix 2. Arrow indicates the site of RNA cleavage. All the ribozymes discussed herein were chemically synthesized by solid phase synthesis using RNA phosphoramadite chemistry, unless otherwise indicated. Those skilled in the art will recognize that these ribozymes could also be made transcriptionally *in vitro* and *in vivo*.

Figure 68 is a graph showing RNA cleavage by hairpin ribozymes targeted to site K. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-K2 (6 bp helix 2) cleaves a 422 target RNA to a greater extent than the HP-K1 (4 bp helix 2).

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 422 nt region (containing hairpin site A) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α-^{32}P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 µl DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1µM) and internally labeled 422 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris·HCl pH 7.5 and 10 mM $MgCl_2$) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 µl were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Figs. 69a-b is the Site L Hairpin Ribozyme (HP-L) showing proposed secondary structure of the hairpin ribozyme•substrate complex. The ribozyme was assembled from two fragments as described above. The nomenclature is the same as above.

Figure 70 shows RNA cleavage by hairpin ribozymes targeted to site L. A. plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-L2 (6 bp helix 2) cleaves a 2 KB target RNA to a greater extent than the HP-L1 (4 bp helix 2). To make internally-labeled substrate RNA for *trans*-ribozyme cleavage reactions, a 2 kB region (containing hairpin site L) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. The cleavage reactions were carried out as described above.

Figs. 71a-b shows a Site M Hairpin Ribozyme (HP-M) with the proposed secondary structure of the hairpin ribozyme•substrate complex. The ribozyme was assembled from two fragments as described above.

Figure 72 is a graph showing RNA cleavage by hairpin ribozymes targeted to site M. The ribozymes were tested at both 20°C and at 26°C. To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.9 KB region (containing hairpin site M) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Cleavage reactions were carried out as described above except that 20°C and at 26°C temperatures were used.

Figs. 73a-d shows various structural modifications of the present invention. A) Hairpin ribozyme lacking helix 5. Nomenclature is same as described under figure 3. B) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by a nucleotide loop wherein q is ≥ 2 bases. Nomenclature is same as described under figure 3. C) Hairpin ribozyme lacking helix 5. Helix 4 loop is replaced by a linker 103"L", wherein L is a non-nucleotide linker molecule (Benseler *et al.*, 1993 *J. Am. Chem. Soc.* 115, 8483; Jennings *et al.*, WO 94/13688). Nomenclature is same as described under figure 3. D) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by non-nucleotide linker molecule "L" (Benseler *et al.*, 1993 *supra*; Jennings *et al.*, *supra*). Nomenclature is same as described under figure 3.

Figs. 74a-b shows Hairpin ribozymes containing nucleotide spacer region "s" at the indicated location, wherein s is ≥ 1 base. Hairpin ribozymes containing spacer region, can be synthesized as one fragment or can be assembled from multiple fragments. Nomenclature is same as described under figure 3.

Figs. 75a-e shows the structures of the 5'-*C*-alkyl-modified nucleotides. R_1 is as defined above. R is OH, H, O-protecting group, NH, or any group described by the publications discussed above, and those described below. B is as defined in the Figure or any other equivalent nucleotide base. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 76 is a diagrammatic representation of the synthesis of 5'-*C*-alkyl-D-**all se** nucleosides and their phosphoramidites.

Figure 77 is a diagrammatic representation of the synthesis of 5'-*C*-alkyl-L-**talose** nucleosides and their phosphoramidites.

Figure 78 is a diagrammatic representation of hammerhead ribozymes targeted to site O containing 5'-C-methyl-L-talo modifications at various positions.

Figure 79 shows RNA cleavage activity of HH-O ribozymes. Fraction of target RNA uncleaved as a function of time is shown.

Figure 80 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* **1992**, *20*, 3252) showing specific substitutions.

Figs. 81a-j shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 82 is a diagrammatic representation of the synthesis of 2'-*C*-allyl uridine and cytidine.

Figure 83 is a diagrammatic representation of the synthesis of 2'-*C*-methylene and 2'-*C*-difluoromethylene uridine.

Figure 84 is a diagrammatic representation of the synthesis of 2'-*C*-methylene and 2'-*C*-difluoromethylene cytidine.

Figure 85 is a diagrammatic representation of the synthesis of 2'-*C*-methylene and 2'-*C*-difluoromethylene adenosine.

Figure 86 is a diagrammatic representation of the synthesis of 2'-*C*-carboxymethylidine uridine, 2'-*C*-methoxycarboxymethylidine uridine and derivatized amidites thereof. X is CH_3 or alkyl as discussed above, or another substituent.

Figure 87 is a diagrammatic representation of a synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Figure 88 is a diagrammatic r presentation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonate 3'-phosphoramidites, dimers and solid supported dimers.

Figure 89 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylene triphosphates.

Figures 90 and 91 are diagrammatic representations of the synthesis of 3'-deoxy-3'-difluoromethylphosphonates and dimers.

Figure 92 is a schematic representation of synthesizing RNA phosphoramidite of a nucleotide containing a 2'-hydroxyl group modification of the present invention.

Figs. 93a-b describes a method for deprotection of oligonucleotides containing a 2'-hydroxyl group modification of the present invention.

Figure 94 is a diagrammatic representation of a hammerhead ribozyme targeted to site N. Positions of 2'-hydroxyl group substitution is indicated.

Figure 95 shows RNA cleavage activity of ribozymes containing a 2'-hydroxyl group modification of the present invention. All RNA, represents hammerhead ribozyme (HHN) with no 2'-hydroxyl group modifications. U7-ala, represents HHN ribozyme containing 2'-NH-alanine modification at the U7 position. U4/U7-ala, represents HHA containing 2'-NH-alanine modifications at U4 and U7 positions. U4 lys, represents HHA containing 2'-NH-lysine modification at U4 position. U7 lys, represents HHA containing 2'-NH-lysine modification at U7 position. U4/U7-lys, represents HHN containing 2'-NH-lysine modification at U4 and U7 positions.

Figures 96 and 97 are schematic representations of synthesizing (solid-phase synthesis) 3' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figure 98 and 99 are schematic representations of synthesizing (solid-phase synthesis) 5' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figures 100 and 101 are g neral schematic representations of the invention.

Fig. 102a-d is a schematic representation of a method of the invention.

Fig. 103 is a graph of the results of the experiment diagrammed in figure 104.

Figure 104 is a diagrammatic representation of a fusion mRNA used in the experiment diagrammed in Fig. 102.

Figure 105 is a diagrammatic representation of a method for selection of useful ribozymes of this invention.

Figure 106 generally shows R-loop formation, and an R-loop complex. In addition, it indicates the location at which ligands can be provided to target the R-loop complex to cells using at least three different procedures, such as ligand receptor interaction, lipid or calcium phosphate mediated delivery, or electroporation.

Figure 107 shows a method for use of self-processing ribozymes to generate therapeutic ribozymes of unit length. This method is essentially described by Draper et al., PCT WO 93/23509.

Figure 108 shows a method of linking ligands like folate, carbohydrate or peptides to R-loop forming RNA.

Ribozymes of this invention block to some extent ICAM-1, IL-5, rel A, TNF-α, p210$^{bcr\text{-}abl}$, or RSV genes expression and can be used to treat diseases or diagnose such diseases. Ribozymes will be delivered to cells in culture and to tissues in animal models. Ribozyme cleavage of ICAM-1, Il-5, rel A, TNF-α ,p210$^{bcr\text{-}abl}$, or RSV mRNA in these systems may prevent or alleviate disease symptoms or conditions.

__I. Target sites__

Targets for useful ribozymes can be determined as disclosed in Draper et al PCT WO93/23509, Sullivan *et al.*, PCT WO94/02595 as well as by Draper et al., PCT/US94/13129 and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as d scribed in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be

optimized and deliv red as described therein. While specific examples to animal and human RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

It must be established that the sites predicted by the computer-based RNA folding algorithm correspond to potential cleavage sites. Hammerhead or hairpin ribozymes are designed that could bind and are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci., USA, 86 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

mRNA is screened for accessible cleavage sites by the method described generally in Draper et al., PCT WO93/23569 hereby incorporated by reference herein. Briefly, DNA oligonucleotides representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to generate a substrate for T7 RNA polymerase transcription from cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from DNA templates. The oligonucleotides and the labeled trascripts are annealed, RNaseH is added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a phosphor imaging system. From these data, hammerhead or hairpin ribozynme sites are chosen as the most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences desribed above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990

Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, phosphoramidites at the 3'-end. The average stepwise coupling yeilds are >98%. Inactive ribozymes are synthesized by substituting a U for G_5 and a U for A_{14} (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbach, 1989, *Methods Enzymol*, 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'H (for a review see Usman and Cedergren, 1992 *TIBS* 17,34). Ribozymes are purified by gel electrophoresis using heneral methods or are purified by high pressure liquid chromatography and are resuspended in water.

Example 1: ICAM-1

Ribozymes that cleave ICAM-1 mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. ICAM-1 function can be blocked therapeutically using monoclonal antibodies. Ribozymes have the advantage of being generally immunologically inert, whereas significant neutralizing anti-IgG responses can be observed with some monoclonal antibody treatments.

The following is a brief description of the physiological role of ICAM-1. The discussion is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein whose expression is induced by inflammatory mediators. ICAM-1 is required for adhesion of leukocytes to endothelial cells and for several immunological functions including antigen presentation, immunoglobulin production and cytotoxic cell activity. Blocking ICAM-1 function prevents immune cell recognition and activity during transplant rejection and in animal models of rheumatoid arthritis, asthma and reperfusion injury.

Cell-cell adhesion plays a pivotal role in inflammatory and immune responses (Springer et al., 1987 *Ann. Rev. Immunol.* 5, 223-252). Cell adhesion is required for leukocytes to bind to and migrate through vascular endothelial cells. In addition, cell-cell adhesion is required for antigen presentation to T cells, for B cell induction by T cells, as well as for the cytotoxicity activity of T cells, NK cells, monocytes or granulocytes. Intercellular adhesion molecule-1 (ICAM-1) is a 110 kilodalton member of the immunoglobulin superfamily that is involved in all of these cell-cell interactions (Simmons et al., 1988 *Nature (London)* 331, 624-627).

ICAM-1 is expressed on only a limited number of cells and at low levels in the absence of stimulation (Dustin et al., 1986 *J. Immunol.* 137, 245-254). Upon treatment with a number of inflammatory mediators (lipopolysaccharide, γ-interferon, tumor necrosis factor-α, or interleukin-1), a variety of cell types (endothelial, epithelial, fibroblastic and hematopoietic cells) in a variety of tissues express high levels of ICAM-1 on their surface (Sringer *et. al. supra;* Dustin *et al., supra;* and Rothlein et al., 1988 *J. Immunol.* 141, 1665-1669). Induction occurs via increased transcription of ICAM-1 mRNA (Simmons *et al., supra*). Elevated expression is detectable after 4 hours and peaks after 16 - 24 hours of induction.

ICAM-1 induction is critical for a number of inflammatory and immune responses. *In vitro*, antibodies to ICAM-1 block adhesion of leukocytes to cytokine-activated endothelial cells (Boyd,1988 *Proc. Natl. Acad. Sci. USA* 85, 3095-3099; Dustin and Springer, 1988 *J. Cell Biol.* 107, 321-331). Thus, ICAM-1 expression may be required for the extravasation of immune cells to sites of inflammation. Antibodies to ICAM-1 also block T cell killing, mixed lymphocyte reactions, and T cell-mediated B cell differentiation, suggesting that ICAM-1 is required for these cognate cell interactions (Boyd *et al., supra)*. The importance of ICAM-1 in antigen presentation is underscored by the inability of ICAM-1 defective murine B cell mutants to stimulate antigen-dependent T cell proliferation (Dang et al., 1990 *J. Immunol.* 144, 4082-4091). Conversely, murine L cells require transfection with human ICAM-1 in addition to HLA-DR in order to present antigen to human T cells (Altmann et al., 1989 *Nature (London)* 338, 512-514). In summary, evidence *in vitro* indicates that ICAM-1 is required for cell-cell interactions critical to inflammatory responses, cellular immune responses, and humoral antibody responses.

By engineering ribozyme motifs we have designed several ribozymes directed against ICAM-1 mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance. These ribozymes cleave ICAM-1 target sequences *in vitro.*

The sequence of human, rat and mouse ICAM-1 mRNA can be screened for accessible sites using a compter folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Tables 2, 3, and 6-9. (All sequences are 5' to 3' in the tables) While rat, mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 4 - 8 and 10. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

The ribozymes will be tested for function *in vivo* by exogenous delivery to human umbilical vein endothelial cells (HUVEC). Ribozymes will be delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors described above. Cytokine-induced ICAM-1 expression will be monitored by ELISA, by indirect immunofluoresence, and/or by FACS analysis. ICAM-1 mRNA levels will be assessed by Northern, by RNAse protection, by primer extension or by quantitative RT-PCR analysis. Ribozymes that block the induction of ICAM-1 protein and mRNA by more than 90% will be identified.

As disclosed by Sullivan et al., PCT WO94/02595, incorporated by reference herein, ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of ICAM-1 mRNA and protein. The effect of the anti-ICAM-1 ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced

into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-ICAM-1 ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

<u>Uses</u>

ICAM-1 plays a central role in immune cell recognition and function. Ribozyme inhibition of ICAM-1 expression can reduce transplant rejection and alleviate symptoms in patients with rheumatoid arthritis, asthma or other acute and chronic inflammatory disorders. We have engineered several ribozymes that cleave ICAM-1 mRNA. Ribozymes that efficiently inhibit ICAM-1 expression in cells can be readily found and their activity measured with regard to their ability to block transplant rejection and arthritis symptoms in animal models. These anti-ICAM-1 ribozymes represent a novel therapeutic for the treatment of immunological or inflammatory disorders.

The therapeutic utility of reduction of activity of ICAM-1 function is evident in the following disease targets. The noted references indicate the role of ICAM-1 and the therapeutic potential of ribozymes described herein. Thus, these targets can be therapeutically treated with agents that reduce ICAM-1 expression or function. These diseases and the studies that support a critical role for ICAM-1 in their pathology are listed below. This list is not meant to be complete and those in the art will recognize further conditions and diseases that can be effectively treated using ribozymes of the present invention.

- Transplant rejection

ICAM-1 is expressed on venules and capillaries of human cardiac biopsies with histological evidence of graft rejection (Briscoe et al., 1991 *Transplantation* 51, 537-539).

Antibody to ICAM-1 blocks renal (Cosimi et al., 1990 *J. Immunol.* 144, 4604-4612) and cardiac (Flavin et al., 1991 *Transplant. Proc.* 23, 533-534) graft rejection in primates.

A Phase I clinical trial of a monoclonal anti-ICAM-1 antibody showed significant reduction in rejection and a significant increase in graft function in human kidney transplant patients (Haug, et al., 1993 *Transplantation* 55, 766-72).

- Rheumatoid arthritis

ICAM-1 overexpression is seen on synovial fibroblasts, endothelial cells, macrophages, and some lymphocytes (Chin et al., 1990 *Arthritis Rheum* 33, 1776-86; Koch et al., 1991 *Lab Invest* 64, 313-20).

Soluble ICAM-1 levels correlate with disease severity (Mason et al., 1993 *Arthritis Rheum* 36, 519-27).

Anti-ICAM antibody inhibits collagen-induced arthritis in mice (Kakimoto et al., 1992 *Cell Immunol* 142, 326-37).

Anti-ICAM antibody inhibits adjuvant-induced arthritis in rats (Iigo et al., 1991 *J Immunol* 147, 4167-71).

- Myocardial ischemia, stroke, and reperfusion injury

Anti-ICAM-1 antibody blocks adherence of neutrophils to anoxic endothelial cells (Yoshida et al., 1992 *Am J Physiol* 262, H1891-8).

Anti-ICAM-1 antibody reduces neurological damage in a rabbit model of cerebral stroke (Bowes et al., 1993 *Exp Neurol* 119, 215-9).

Anti-ICAM-1 antibody protects against reperfusion injury in a cat model of myocardial ischemia (Ma et al., 1992 *Circulation* 86, 937-46).

- Asthma

Antibody to ICAM-1 partially blocks eosinophil adhesion to endothelial cells and is overexpressed on inflamed airway endothelium and epithelium *in vivo* (Wegner et al., 1990 *Science* 247, 456-9).

In a primate model of asthma, anti-ICAM-1 antibody blocks airway eosinophilia (Wegneret al., *supra*) and prevents the resurgence of airway inflammation and hyper-responsiveness after dexamethosone treatment (Gundel et al., 1992 *Clin Exp Allergy* 22, 569-75).

- Psoriasis

Surface ICAM-1 and a clipped, soluble version of ICAM-1 is expressed in psoriatic lesions and expression correlates with inflammation (Kellner et al., 1991 *Br J Dermatol* 125, 211-6; Griffiths 1989 *J Am Acad Dermatol* 20, 617-29; Schopf et al., 1993 *Br J Dermatol* 128, 34-7).

Anti-ICAM antibody blocks keratinocyte antigen presentation to T cells (Nickoloff et al., 1993*J Immunol* 150, 2148-59).

- Kawasaki disease

Surface ICAM-1 expression correlates with the disease and is reduced by effective immunoglobulin treatment (Leung, et al., 1989*Lancet* 2, 1298-302).

Soluble ICAM levels are elevated in Kawasaki disease patients; particularly high levels are observed in patients with coronary artery lesions (Furukawa et al., 1992*Arthritis Rheum* 35, 672-7; Tsuji, 1992 *Arerugi* 41, 1507-14).

Circulating LFA-1^+ T cells are depleted (presumably due to ICAM-1 mediated extravasation) in Kawasaki disease patients (Furukawa et al., 1993*Scand J Immunol* 37, 377-80).

Example 2: IL-5

Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to inflammatory disorders like asthma. The invention features use of ribozymes to treat chronic asthma, e.g., by inhibiting the synthesis of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

A number of cytokines besides IL-5 may also be involved in the activation of inflammation in asthmatic patients, including platelet activating factor, IL-1, IL-3, IL-4, GM-CSF, TNF-α, gamma interferon, VCAM, ILAM-1, ELAM-1 and NF-κB. In addition to these molecules, it is appreciated that any cellular receptors which mediate the activities of the cytokines are also good targets for intervention in inflammatory diseases. These targets include, but are not limited to, the IL-1R and TNF-αR on keratinocytes, epithelial and endothelial cells in airways. Recent data suggest that certain neuropeptides may play a role in asthmatic symptoms. These peptides include substance P, neurokinin A and calcitonin-gen -related peptides. These target genes may have more general roles in inflammatory diseases, but are currently assumed to have a role only in asthma.

Ribozymes of this invention block to some extent IL-5 expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of asthma (Clutterbuck et al., 1989 supra; Garssen et al., 1991 Am. Rev. Respir. Dis. 144, 931-938; Larsen et al., 1992 J. Clin. Invest. 89, 747-752; Mauser et al., 1993 supra). Ribozyme cleavage of IL-5 mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse IL-5 mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 11, 13, and 14, 15. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 12, lower case letters indicate positions that are not conserved between the Human and the Mouse IL-5 sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 12, 14 - 16. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables 12 and 14 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 15 and 16 (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 12, 14 - 16 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against IL-5 mRNA sequences. These ribozymes are synthesized

with modifications that improve their nuclease resistance. The ability of ribozymes to cl ave IL-5 target sequences *in vitro* is evaluated.

The ribozymes will be tested for function *in vivo* by analyzing IL-5 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors. IL-5 expression will be monitored by biological assays, ELISA, by indirect immunofluoresence, and/or by FACS analysis. IL-5 mRNA levels will be assessed by Northern analysis, RNAse protection or primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of IL-5 activity and/or IL-5 mRNA by more than 90% will be identified.

Uses

Interleukin 5 (IL-5), a cytokine produced by CD4+ T helper cells and mast cells, was originally termed B cell growth factor II (reviewed by Takatsu et al., 1988 Immunol. Rev. 102, 107). It stimulates proliferation of activated B cells and induces production of IgM and IgA. IL-5 plays a major role in eosinophil function by promoting differentiation (Clutterbuck et al., 1989 Blood 73, 1504-12), vascular adhesion (Walsh et al., 1990 Immunology 71, 258-65) and *in vitro* survival of eosinophils (Lopez et al., 1988 J. Exp. Med. 167, 219-24). This cytokine also enhances histamine release from basophils (Hirai et al., 1990 J. Exp. Med. 172, 1525-8). The following summaries of clinical results support the selection of IL-5 as a primary target for the treatment of asthma:

Several studies have shown a direct correlation between the number of activated T cells and the number of eosinophils from asthmatic patients vs. normal patients (Oehling et al., 1992 J. Investig. Allergol. Clin. Immunol. 2, 295-9). Patients with either allergic asthma or intrinsic asthma were treated with corticosteroids. The bronchoalveolar lavage was monitored for eosinophils, activated T helper cells and recovery of pulmonary function over a 28 to 30 day period. The number of eosinophils and activated T helper cells decreased progressively with subsequent improvement in pulmonary function compared to intrinsic asthma patients with no corticosteroid treatment.

Bronchoalv olar lavage cells were screened for production of cytokines using *in situ* hybridization for mRNA. *In situ* hybridization signals

were detected for IL-2, IL-3, IL-4, IL-5 and GM-CSF. Upregulation of mRNA was observed for IL-4, IL-5 and GM-CSF (Robinson et al., 1993 J. Allergy Clin. Immunol. 92, 313-24). Another study showed that upregulation of IL-5 transcripts from allergen challenged vs. saline challenged asthmatic patients (Krishnaswamy et al., 1993 Am. J. Respir. Cell. Mol. Biol. 9, 279-86).

An 18 patient study was performed to determine a mechanism of action for corticosteroid improvement of asthma symptoms. Improvement was monitored by methacholine responsiveness. A correlation was observed between the methacholine responsiveness, a reduction in the number of eosinophils, a reduction in the number of cells expressing IL-4 and IL-5 mRNA and an increase in number of cells expressing interferon-gamma.

Bronchial biopsies from 15 patients were analyzed 24 hours after allergen challenge (Bentley et al., 1993 Am. J. Respir. Cell. Mol. Biol. 8, 35-42). Increased numbers of eosinophils and IL-2 receptor positive cells were found in the biopsies. No differences in the numbers of total leukocytes, T lymphocytes, elastase-positive neutrophils, macrophages or mast cell subtypes were observed. The number of cells expressing IL-5 and GM-CSF mRNA significantly increased.

In another patient study, the eosinophil phenotype was the same for asthmatic patients and normal individuals. However, eosinophils from asthmatic patients had greater leukotriene C4 producing capacity and migration capacity. There were elevated levels of IL-3, IL-5 and GM-CSF in the circulation of asthmatics but not in normal individuals (Bruijnzeel et al., 1992 Schweiz. Med. Wochenschr. 122, 298-301).

Efficacy of antibody to IL-5 was assessed in a guinea pig asthma model. The animals were challenged with ovalbumin and assayed for eosinophilia and the responsiveness to the bronchioconstriction substance P. A 30 mg/kg dose of antibody administered i.p. blocked ovalbumin-induced increased sensitivity to substance P and blocked increases in bronchoalveolar and lung tissue accumulation of eosinophils (Mauser et al., 1993 Am. Rev. Respir. Dis. 148, 1623-7). In a separate study guinea pigs challenged for eight days with ovalbumin were treated with monoclonal antibody to IL-5. Treatment produced a reduction in the

number of eosinophils in bronchoalveolar lavage. No reduction was observed for unchallenged guinea pigs and guinea pigs treated with a control antibody. Antibody treatment completely inhibited the development of hyperreactivity to histamine and arecoline after ovalbumin challenge (van Oosterhout et al., 1993 Am. Rev. Respir. Dis. 147, 548-52)

Results obtained from human clinical analysis and animal studi s indicate the role of activated T helper cells, cytokines and eosinophils in asthma. The role of IL-5 in eosinophil development and function makes IL-5 a good candidate for target selection. The antibody studies neutralized IL-5 in the circulation thus preventing eosinophilia. Inhibition of the production of IL-5 will achieve the same goal.

Asthma – a prominent feature of asthma is the infiltration of eosinophils and deposition of toxic eosinophil proteins (e.g. major basic protein, eosinophil-derived neurotoxin) in the lung. A number of T-cell-derived factors like IL-5 are responsible for the activation and maintainance of eosinophils (Kay, 1991 J. Allergy Clin. Immun. 87, 893). Inhibition of IL-5 expression in the lungs can decrease the activation of eosinophils and will help alleviate the symptoms of asthma.

Atopy – is characterized by the developement of type I hypersensitive reactions associated with exposure to certain environmental antigens. One of the common clinical manifestations of atopy is eosinophilia (accumulation of abnormally high levels of eosinophils in the blood). Antibodies against IL-5 have been shown to lower the levels of eosinophils in mice (Cook et al., 1993 in Immunopharmacol. Eosinophils ed. Smith and Cook, pp. 193-216, Academic, London, UK)

Parasitic infection-related eosinophilia– infections with parasites like helminths, can lead to severe eosinophilia (Cook et al., 1993 supra). Animal models for eosinophilia suggest that infection of mice, for example, can lead to blood, peritoneal and/or tissue eosinophilia, all of which seem to be lowered to varying degrees by antibodies directed against IL-5.

Pulmonary infiltration eosinophilia– is characterised by accumulation of high levels of eosinophils in pulmonary parenchyma (Gleich, 1990 J. Allergy Clin. Immunol. 85, 422).

L-Trypt phan-ass ciated osin philia-myalgia syndrome (EMS)– The EMS disease is closely linked to the consumption of L-tryptophan, an essential aminoacid used to treat conditions like insomnia (for review see Varga et al., 1993 J Invest. Dermatol. 100, 97s). Pathologic and histologic studies have demonstrated high levels of eosinophils and mononuclear inflammatory cells in patients with EMS. It appears that IL-5 and transforming growth factor play a significant role in the development of EMS (Varga et al., 1993 supra) by activating eosinophils and other inflammatory cells.

Thus, ribozymes of the present invention that cleave IL-5 mRNA and thereby IL-5 activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits IL-5 function is described above; available cellular and activity assays are numerous, reproducible, and accurate. Animal models for IL-5 function and for each of the suggested disease targets exist (Cook et al., 1993 supra) and can be used to optimize activity.

Example 3: NF–κB

Ribozymes that cleave *rel A* mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. Inflammatory mediators such as lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor-a (TNF-α) act on cells by inducing transcription of a number of secondary mediators, including other cytokines and adhesion molecules. In many cases, this gene activation is known to be mediated by the transcriptional regulator, NF–κB. One subunit of NF–κB, the *rel*A gene product (termed RelA or p65) is implicated specifically in the induction of inflammatory responses. Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target intracellular factors that contribute to disease pathology. Thus, ribozymes that cleave mRNA encoded by rel A or TNF-α may represent novel therapeutics for the treatment of inflammatory and autoimmune disorders.

The nuclear DNA-binding activity, NF–κB, was first identified as a factor that binds and activates the immunoglobulin κ light chain enhancer in B cells. NF–κB now is known to activate transcription of a variety of other cellular genes (*e.g.*, cytokines, adhesion proteins, oncogenes and viral

proteins) in response to a variety of stimuli (*e.g.*, phorbol esters, mitogens, cytokines and oxidative stress). In addition, molecular and biochemical characterization of NF-κB has shown that the activity is due to a homodimer or heterodimer of a family of DNA binding subunits. Each subunit bears a stretch of 300 amino acids that is homologous to the oncogene, v-*rel*. The activity first described as NF-κB is a heterodimer of p49 or p50 with p65. The p49 and p50 subunits of NF-κB (encoded by the nf-κB2 or nf-κB1 genes, respectively) are generated from the precursors NF-κB1 (p105) or NF-κB2 (p100). The p65 subunit of NF-κB (now termed Rel A) is encoded by the *rel* A locus.

The roles of each specific transcription-activating complex now are being elucidated in cells (N.D. Perkins, et al., 1992 Proc. Natl Acad. Sci USA 89, 1529-1533). For instance, the heterodimer of NF-κB1 and Rel A (p50/p65) activates transcription of the promoter for the adhesion molecule, VCAM-1, while NF-κB2/RelA heterodimers (p49/p65) actually inhibit transcription (H.B. Shu, et al., Mol. Cell. Biol. 13, 6283-6289 (1993)). Conversely, heterodimers of NF-κB2/RelA (p49/p65) act with Tat-I to activate transcription of the HIV genome, while NF-κB1/RelA (p50/p65) heterodimers have little effect (J. Liu, N.D. Perkins, R.M. Schmid, G.J. Nabel, J. Virol. 1992 66, 3883-3887). Similarly, blocking *rel* A gene expression with antisense oligonucleotides specifically blocks embryonic stem cell adhesion; blocking NF-κB1 gene expression with antisense oligonucleotides had no effect on cellular adhesion (Narayanan et al., 1993 Mol. Cell. Biol. 13, 3802-3810). Thus, the promiscuous role initially assigned to NF-κB in transcriptional activation (M.J. Lenardo, D. Baltimore, 1989 Cell 58, 227-229) represents the sum of the activities of the rel family of DNA-binding proteins. This conclusion is supported by recent transgenic "knock-out" mice of individual members of the *rel* family. Such "knock-outs" show few developmental defects, suggesting that essential transcriptional activation functions can be performed by more than one member of the rel family.

A number of specific inhibitors of NF-κB function in cells exist, including treatment with phosphorothioate antisense oliogonucleotide, treatment with double-stranded NF-κB binding sites, and over expression of the natural inhibitor MAD-3 (an IκB family member). These agents have

been used to show that NF–κB is requir d for induction of a number of molecules involved in inflammation, as described below.

•NF–κB is required for phorbol ester-mediated induction of IL-6 (I. Kitajima, et al., Science 258, 1792-5 (1992)) and IL-8 (Kunsch and Rosen, 1993 Mol. Cell. Biol. 13, 6137-46).

•NF–κB is required for induction of the adhesion molecules ICAM-1 (Eck, et al., 1993 Mol. Cell. Biol. 13, 6530-6536), VCAM-1 (Shu et al., *supra*), and E-selectin (Read, et al., 1994 J. Exp. Med. 179, 503-512) on endothelial cells.

•NF–κB is involved in the induction of the integrin subunit, CD18, and other adhesive properties of leukocytes (Eck et al., 1993 *supra*).

The above studies suggest that NF–κB is integrally involved in the induction of cytokines and adhesion molecules by inflammatory mediators. Two recent papers point to another connection between NF–κB and inflammation: glucocorticoids may exert their anti-inflammatory effects by inhibiting NF–κB. The glucocorticoid receptor and p65 both act at NF–κB binding sites in the ICAM-1 promoter (van de Stolpe, et al., 1994 J. Biol. Chem. 269, 6185-6192). Glucocorticoid receptor inhibits NF–κB-mediated induction of IL-6 (Ray and Prefontaine, 1994 Proc. Natl Acad. Sci USA 91, 752-756). Conversely, overexpression of p65 inhibits glucocorticoid induction of the mouse mammary tumor virus promoter. Finally, protein cross-linking and co-immunoprecipitation experiments demonstrated direct physical interaction between p65 and the glucocorticoid receptor (*Id.*).

Ribozymes of this invention block to some extent NF–κB expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of restenosis, transplant rejection and rheumatoid arthritis. Ribozyme cleavage of *relA* mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse *rel*A mRNA can be screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 17, 18 and 21-22. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and

ribozymes thereafter designed, the human targetted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 19 - 22. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *rel* A mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave *rel*A target sequences *in vitro* is evaluated.

The ribozymes will be tested for function *in vivo* by analyzing cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA and RNA vectors. Cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression will be monitored by ELISA, by indirect immunofluoresence, and/or by FACS analysis. *Rel* A mRNA levels will be assessed by Northern analysis, RNAse protection or primer extension analysis or quantitative RT-PCR. Activity of NF-κB will be monitored by gel-retardation assays. Ribozymes that block the induction of NF-κB activity and/or *rel A* mRNA by more than 50% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of VCAM-1, ICAM-1, IL-6 and IL-8 mRNA and protein. The effect of the anti-*rel A* ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-*relA* ribozyme or a gene construct that constitutively expresses th ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves *rel A* mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

•Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Restenosis.

Expression of NF-κB in the vessel wall of pigs causes a narrowing of the luminal space due to excessive deposition of extracellular matrix components. This phenotype is similar to matrix deposition that occurs subsequent to coronary angioplasty. In addition, NF-κB is required for the expression of the oncogene c-myb (F.A. La Rosa, J.W. Pierce, G.E. Sonenshein, Mol. Cell. Biol. 14, 1039-44 (1994)). Thus NF-κB induces smooth muscle proliferation and the expression of excess matrix components: both processes are thought to contribute to reocclusion of vessels after coronary angioplasty.

•Transplantation.

NF-κB is required for the induction of adhesion molecules (Eck et al., *supra*, K. O'Brien, et al., J. Clin. Invest. 92, 945-951 (1993)) that function in immune recognition and inflammatory responses. At least two potential modes of treatment are possible. In the first, transplanted organs are treated *ex vivo* with ribozymes or ribozyme expression vectors. Transient inhibition of NF-κB in the transplanted endothelium may be sufficient to prevent transplant-associated vasculitis and may significantly modulate graft rejection. In the second, donor B cells are treated *ex vivo* with ribozymes or ribozyme expression vectors. Recipients would receive the treatment prior to transplant. Treatment of a recipient with B cells that do not express T cell co-stimulatory molecules (such as ICAM-1, VCAM-1, and/or B7 an B7-2) can induce antigen-specific anergy. Tolerance to the donor's histocompatibility antigens could result; potentially, any donor could be used for any transplantation procedure.

•Asthma.

Granulocyte macrophage colony stimulating factor (GM-CSF) is thought to play a major role in recruitment of eosinophils and other inflammatory cells during the late phase reaction to asthmatic trauma. Again, blocking the local induction of GM-CSF and other inflammatory mediators is likely to reduce the persistent inflammation observed in chronic asthmatics. Aerosol delivery of ribozymes or adenovirus ribozyme expression vectors is a feasible treatment.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave *rel A* mRNA and thereby NF-κB activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits NF-κB

function is described above; availabl cellular and activity assays are number, reproducible, and accurate. Animal models for NF–κB function (Kitajima, et al., *supra*) and for each of the suggested disease targets exist and can be used to optimize activity.

Example 4: TNF-α

Ribozymes that cleave the specific cites in TNF-α mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders.

Tumor necrosis factor-α (TNF-α) is a protein, secreted by activated leukocytes, that is a potent mediator of inflammatory reactions. Injection of TNF-α into experimental animals can simulate the symptoms of systemic and local inflammatory diseases such as septic shock or rheumatoid arthritis.

TNF-α was initially described as a factor secreted by activated macrophages which mediates the destruction of solid tumors in mice (Old, 1985 Science 230, 4225-4231). TNF-α subsequently was found to be identical to cachectin, an agent responsible for the weight loss and wasting syndrome associated with tumors and chronic infections (Beutler, et al., 1985 Nature 316, 552-554). The cDNA and the genomic locus for TNF-α have been cloned and found to be related to TNF-ß (Shakhov et al., 1990 J. Exp. Med. 171, 35-47). Both TNF-α and TNF-ß bind to the same receptors and have nearly identical biological activities. The two TNF receptors have been found on most cell types examined (Smith, et al., 1990 Science 248, 1019-1023). TNF-α secretion has been detected from monocytes/macrophages, CD4+ and CD8+ T-cells, B-cells, lymphokine activated killer cells, neutrophils, astrocytes, endothelial cells, smooth muscle cells, as well as various non-hematopoietic tumor cell lines (for a review see Turestskaya et al., 1991 in Tumor Necrosis Factor: Structure, Function, and Mechanism of Action B. B. Aggarwal, J. Vilcek, Eds. Marcel Dekker, Inc., pp. 35-60). TNF-α is regulated transcriptionally and translationally, and requires proteolytic processing at the plasma membrane in order to be secreted (Kriegler et al., 1988 Cell 53, 45-53). Once secreted, the serum half life of TNF-α is approximately 30 minutes. The tight regulation of TNF-α is important due to the extreme toxicity of this cytokine. Increasing evidence indicates that overproduction of TNF-α

during infections can lead to severe systemic toxicity and death (Tracey & Cerami, 1992 Am. J. Trop. Med. Hyg. 47, 2-7).

Antisense RNA and Hammerhead ribozymes have been used in an attempt to lower the expression level of TNF-α by targeting specified cleavage sites [Sioud et al., 1992 J. Mol. Biol. 223; 831; Sioud WO 94/10301; Kisich and co-workers, 1990 abstract (FASEB J. 4, A1860; 1991 slide presentation (J. Leukocyte Biol. sup. 2, 70); December, 1992 poster presentation at Anti-HIV Therapeutics Conference in SanDiego, CA; and "Development of anti-TNF-α ribozymes for the control of TNF-α gene expression"- Kisich, Doctoral Dissertation, 1993 University of California, Davis] listing various TNFα targeted ribozymes.

Ribozymes of this invention block to some extent TNF-α expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of septic shock and rheumatoid arthritis. Ribozyme cleavage of TNF-α mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse TNF-α mRNA can be screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 23, 25, and 27 - 28. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 24, lower case letters indicate positions that are not conserved between the human and the mouse TNF-α sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 24, 26 - 28. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 24 and 26 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any

sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 27 and 28 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 24, 26 - 28 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables or AAV .

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF-α RNA is inserted into a plasmid DNA vector or an adenovirus DNA viral vector or AAV or alpha virus or retroviris vectors. Viral vectors have been used to transfer genes to the intact vasculature or to joints of live animals (Willard et al., 1992 Circulation, 86, I-473.; Nabel et al., 1990 Science, 249, 1285-1288) and both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, *e.g.,* through the use of an injection catheter, stent or infusion pump or are directly added to cells or tissues *ex vivo.*

In another preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF-∝ RNA is inserted into a retrovirus vector for sustained expression of ribozyme(s).

By engineering ribozyme motifs we have designed several ribozymes directed against TNF-α mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave TNF-α target sequences *in vitro* is evaluated.

The ribozymes will be tested for function in cells by analyzing bacterial lipopolysaccharide (LPS)-induced TNF-α expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. TNF-α expression will be monitored by ELISA, by indirect immunofluoresence, and/or by FACS analysis. TNF-α mRNA levels will be assessed by Northern analysis, RNAse protection, primer extension

analysis or quantitative RT-PCR. Ribozymes that block the induction of TNF-α activity and/or TNF-α mRNA by more than 90% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to macrophages by intraperitoneal injection. After a period of ribozyme uptake, the peritoneal macrophages are harvested and induced *ex vivo* with LPS. The ribozymes that significantly reduce TNF-α secretion are selected. The TNF-α can also be induced after ribozyme treatment with fixed *Streptococcus* in the peritoneal cavity instead of *ex vivo*. In this fashion the ability of TNF-α ribozymes to block TNF-α secretion in a localized inflammatory response are evaluated. In addition, we will determine if the ribozymes can block an ongoing inflammatory response by delivering the TNF-α ribozymes after induction by the injection of fixed *Streptococcus.*

To examine the effect of anti-TNF-α ribozymes on systemic inflammation, the ribozymes are delivered by intravenous injection. The ability of the ribozymes to inhibit TNF-α secretion and lethal shock caused by systemic LPS administration are assessed. Similarly, TNF-α ribozymes can be introduced into the joints of mice with collagen-induced arthritis. Either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments can be used to supply ribozymes. One dose (or a few infrequent doses) of a stable anti-TNF-α ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate tissue damage in these inflammatory diseases.

Macrophage isolation.

To produce responsive macrophages 1 ml of sterile fluid thioglycollate broth (Difco, Detroit, MI.) was injected i.p. into 6 week old female C57bl/6NCR mice 3 days before peritoneal lavage. Mice were maintained as specific pathogen free in autoclaved cages in a laminar flow hood and given sterilized water to minimize "spontaneous" activation of macrophages. The resulting peritoneal exudate cells (PEC) were obtained by lavage using Hanks balanced salt solution (HBSS) and were plated at $2.5X10^5$/well in 96 well plates (Costar, Cambridge, MA.) with Eagles minimal essential medium (EMEM) containing 10% heat inactivated fetal

bovine serum. After adhering for 2 hours the wells were washed to remove non-adherent cells. The resulting cultures were 97% macrophages as determined by morphology and staining for non-specific esterase.

Transfection of ribozymes into macrophages:

The ribozymes were diluted to 2X final concentration, mixed with an equal volume of 11nM lipofectamine (Life Technologies, Gaithersburg, MD.), and vortexed. 100 ml of lipid:ribozyme complex was then added directly to the cells, followed immediately by 10 ml fetal bovine serum. Three hours after ribozyme addition 100 ml of 1 mg/ml bacterial lipopolysaccaride (LPS) was added to each well to stimulate TNF production.

Quantitation of TNF-α in mouse macrophages:

Supernatants were sampled at 0, 2, 4, 8, and 24 hours post LPS stimulation and stored at -70^{o}C. Quantitation of TNF-α was done by a specific ELISA. ELISA plates were coated with rabbit anti-mouse TNF-α serum at 1:1000 dilution (Genzyme) followed by blocking with milk proteins and incubation with TNF-α containing supernatants. TNF-α was then detected using a murine TNF-α specific hamster monoclonal antibody (Genzyme). The ELISA was developed with goat anti-hamster IgG coupled to alkaline phosphatase.

Assessment of reagent toxicity:

Following ribozyme/lipid treatment of macrophages and harvesting of supernatants viability of the cells was assessed by incubation of the cells with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). This compound is reduced by the mitochondrial dihydrogenases, the activity of which correlates well with cell viability. After 12 hours the absorbance of reduced MTT is measured at 585 nm.

Uses

The association between TNF-α and bacterial sepsis, rheumatoid arthritis, and autoimmune disease make TNF-α an attractive target for therapeutic intervention [Tracy & Cerami 1992 supra; Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788; Jacob, 1992 J. Autoimmun. 5 (Supp. A), 133-143].

Septic Shock

Septic shock is a complication of major surgery, bacterial infection, and polytrauma characterized by high fever, increased cardiac output, reduced blood pressure and a neutrophilic infiltrate into the lungs and other major organs. Current treatment options are limited to antibiotics to reduce the bacterial load and non-steroidal anti-inflammatories to reduce fever. Despite these treatments in the best intensive care settings, mortality from septic shock averages 50%, due primarily to multiple organ failure and disseminated vascular coagulation. Septic shock, with an incidence of 200,000 cases per year in the United States, is the major cause of death in intensive care units. In septic shock syndrome, tissue injury or bacterial products initiate massive immune activation, resulting in the secretion of pro-inflammatory cytokines which are not normally detected in the serum, such as TNF-α, interleukin-1ß (IL-1ß), γ-interferon (IFN-γ), interleukin-6 (IL-6), and interleukin-8 (IL-8). Other non-cytokine mediators such as leukotriene b4, prostaglandin E2, C3a and C3d also reach high levels (de Boer et al., 1992 Immunopharmacology 24, 135-148).

TNF-α is detected early in the course of septic shock in a large fraction of patients (de Boer et al., 1992 supra). In animal models, injection of TNF-α has been shown to induce shock-like symptoms similar to those induced by LPS injection (Beutler et al., 1985 Science 229, 869-871); in contrast, injection of IL-1ß, IL-6, or IL-8 does not induce shock. Injection of TNF-α also causes an elevation of IL-1ß, IL-6, IL-8, PgE_2, acute phase proteins, and TxA_2 in the serum of experimental animals (de Boer et al., 1992 supra). In animal models the lethal effects of LPS can be blocked by pre-administration of anti-TNF-α antibodies. The cumulative evidence indicates that TNF-α is a key player in the pathogenesis of septic shock, and therefore a good candidate for therapeutic intervention.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints leading to bone destruction and loss of joint function. At the cellular level, autoreactive T- lymphocytes and monocytes are typically present, and the synoviocytes often have altered morphology and immunostaining patterns. RA joints have been shown to contain elevated levels of TNF-α, IL-1α and IL-1ß, IL-6, GM-CSF, and TGF-

ß (Abney et al., 1991 Imm. Rev. 119, 105-123), some or all of which may contribute to the pathological course of the disease.

Cells cultured from RA joints spontaneously secrete all of the pro-inflammatory cytokines detected *in vivo*. Addition of antisera against TNF-α to these cultures has been shown to reduce IL-1α/ß production by these cells to undetectable levels (Abney et al., 1991 Supra). Thus, TNF-α may directly induce the production of other cytokines in the RA joint. Addition of the anti-inflammatory cytokine, TGF-ß, has no effect on cytokine secretion by RA cultures. Immunocytochemical studies of human RA surgical specimens clearly demonstrate the production of TNF-α, IL-1α/ß, and IL-6 from macrophages near the cartilage/pannus junction when the pannus in invading and overgrowing the cartilage (Chu et al., 1992 Br. J. Rheumatology 31, 653-661). GM-CSF was shown to be produced mainly by vascular endothelium in these samples. Both TNF-α and TGF-ß have been shown to be fibroblast growth factors, and may contribute to the accumulation of scar tissue in the RA joint. TNF-α has also been shown to increase osteoclast activity and bone resorbtion, and may have a role in the bone erosion commonly found in the RA joint (Cooper et al., 1992 Clin. Exp. Immunol. 89, 244-250).

Elimination of TNF-α from the rheumatic joint would be predicted to reduce overall inflammation by reducing induction of MHC class II, IL-1α/ß, Il-6, and GM-CSF, and reducing T-cell activation. Osteoclast activity might also fall, reducing the rate of bone erosion at the joint. Finally, elimination of TNF-α would be expected to reduce accumulation of scar tissue within the joint by removal of a fibroblast growth factor.

Treatment with an anti-TNF-α antibody reduces joint swelling and the histological severity of collagen-induced arthritis in mice (Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788). In addition, a study of RA patients who have received i.v. infusions of anti-TNF-α monoclonal antibody reports a reduction in the number and severity of inflamed joints after treatment. The benefit of monoclonal antibody treatment in the long term may be limited by the expense and immunogenicity of the antibody.

Psoriasis

Psoriasis is an inflammatory disorder of the skin characterized by keratinocyte hyperproliferation and immune cell infiltrate (Kupper, 1990 J.

Clin. Invest. 86, 1783-1789). It is a fairly common condition, affecting 1.5-2.0% of the population. The disorder ranges in severity from mild, with small flaky patches of skin, to severe, involving inflammation of the entire epidermis. The cellular infiltrate of psoriasis includes T-lymphocytes, neutrophils, macrophages, and dermal dendrocytes. The majority of T-lymphocytes are activated $CD4^+$ cells of the T_H-1 phenotype, although some $CD8^+$ and $CD4^-/CD8^-$ are also present. B lymphocytes are typically not found in abundance in psoriatic plaques.

Numerous hypotheses have been offered as to the proximal cause of psoriasis including auto-antibodies and auto-reactive T-cells, overproduction of growth factors, and genetic predisposition. Although there is evidence to support the involvement of each of these factors in psoriasis, they are neither mutually exclusive nor are any of them necessary and sufficient for the pathogenesis of psoriasis (Reeves, 1991 Semin. Dermatol. 10, 217).

The role of cytokines in the pathogenesis of psoriasis has been investigated. Among those cytokines found to be abnormally expressed were TGF-α , IL-1α, IL-1ß, IL-1ra, IL-6, IL-8, IFN-γ, and TNF-α . In addition to abnormal cytokine production, elevated expression of ICAM-1, ELAM-1, and VCAM has been observed (Reeves, 1991 supra). This cytokine profile is similar to that of normal wound healing, with the notable exception that cytokine levels subside upon healing. Keratinocytes themselves have recently been shown to be capable of secreting EGF, TGF-α, IL-6, and TNF-α, which could increase proliferation in an autocrine fashion (Oxholm et al., 1991 APMIS 99, 58-64).

Nickoloff et al., 1993 (J Dermatol Sci. 6, 127-33) have proposed the following model for the initiation and maintenance of the psoriatic plaque:

Tissue damage induces the wound healing response in the skin. Keratinocytes secrete IL-1α, IL-1ß, IL-6, IL-8, TNF-α. These factors activate the endothelium of dermal capillaries, recruiting PMNs, macrophages, and T-cells into the wound site.

Dermal dendrocytes near the dermal/epidermal junction remain activated when they should return to a quiescent state, and subsequently secrete cytokines including TNF-α, IL-6, and IL-8. Cytokine expression, in

turn, maintains the activated state of the endothelium, allowing extravasation of additional immunocytes, and the activated state of the keratinocytes which secrete TGF-α and IL-8. Keratinocyte IL-8 recruits immunocytes from the dermis into the epidermis. During passage through the dermis, T-cells encounter the activated dermal dendrocytes which efficiently activate the T_H-1 phenotype. The activated T-cells continue to migrate into the epidermis, where they are stimulated by keratinocyte-expressed ICAM-1 and MHC class II. IFN-γ secreted by the T-cells synergizes with the TNF-α from dermal dendrocytes to increase keratinocyte proliferation and the levels of TGF-α, IL-8, and IL-6 production. IFN-γ also feeds back to the dermal dendrocyte, maintaining the activated phenotype and the inflammatory cycle.

Elevated serum titres of IL-6 increases synthesis of acute phase proteins including complement factors by the liver, and antibody production by plasma cells. Increased complement and antibody levels increases the probability of autoimmune reactions.

Maintenance of the psoriatic plaque requires continued expression of all of these processes, but attractive points of therapeutic intervention are TNF-α expression by the dermal dendrocyte to maintain activated endothelium and keratinocytes, and IFN-γ expression by T-cells to maintain activated dermal dendrocytes.

There are 3 million patients in the United States afflicted with psoriasis. The available treatments for psoriasis are corticosteroids. The most widely prescribed are TEMOVATE (clobetasol propionate), LIDEX (fluocinonide), DIPROLENE (betamethasone propionate), PSORCON (diflorasone diacetate) and TRIAMCINOLONE formulated for topical application. The mechanism of action of corticosteroids is multifactorial. This is a palliative therapy because the underlying cause of the disease remains, and upon discontinuation of the treatment the disease returns. Discontinuation of treatment is often prompted by the appearance of adverse effects such as atrophy, telangiectasias and purpura. Corticosteroids are not recommended for prolonged treatments or when treatment of large and/or inflamed areas is required. Alternative treatments include retinoids, such as etretinate, which has been approved for treatment of severe, refractory psoriasis. Alternative retinoid-based treatments are in advanced clinical trials. Retinoids act by converting

keratinocytes to a differentiated state and restoration of normal skin development. Immunosuppressive drugs such as cyclosporine are also in the advanced stages of clinical trials. Due to the nonspecific mechanism of action of corticosteroids, retinoids and immunosuppressives, these treatments exhibit severe side effects and should not be used for extended periods of time unless the condition is life-threatening or disabling. There is a need for a less toxic, effective therapeutic agent in psoriatic patients.

<u>HIV and AIDS</u>

The human immunodeficiency virus (HIV) causes several fundamental changes in the human immune system from the time of infection until the development of full-blown acquired immunodeficiency syndrome (AIDS). These changes include a shift in the ratio of CD4+ to CD8+ T-cells, sustained elevation of IL-4 levels, episodic elevation of TNF-α and TNF-β levels, hypergammaglobulinemia, and lymphoma/leukemia (Rosenberg & Fauci, 1990 <u>Immun. Today</u> 11, 176; Weiss 1993 <u>Science</u> 260, 1273). Many patients experience a unique tumor, Kaposi's sarcoma and/or unusual opportunistic infections (e.g. *Pneumocystis carinii*, cytomegalovirus, herpesviruses, hepatitis viruses, papilloma viruses, and tuberculosis). The immunological dysfunction of individuals with AIDS suggests that some of the pathology may be due to cytokine dysregulation.

Levels of serum TNF-α and IL-6 are often found to be elevated in AIDS patients (Weiss, 1993 <u>supra</u>). In tissue culture, HIV infection of monocytes isolated from healthy individuals stimulates secretion of both TNF-α and IL-6. This response has been reproduced using purified gp120, the viral coat protein responsible for binding to CD-4 (Buonaguro et al., 1992 <u>J. Virol.</u> 66, 7159). It has also been demonstrated that the viral gene regulator, Tat, can directly induce TNF transcription. The ability of HIV to directly stimulate secretion of TNF-α and IL-6 may be an adaptive mechanism of the virus. TNF-α has been shown to upregulate transcription of the LTR of HIV, increasing the number of HIV-specific transcripts in infected cells. IL-6 enhances HIV production, but at a post-transcriptional level, apparently increasing the efficiency with which HIV transcripts are translated into protein. Thus, stimulation of TNF-α secretion by the HIV virus may promote infection of neighboring CD4+ cells both by enhancing virus production from latently infected cells and by driving replication of the virus in newly infected cells.

The role of TNF-α in HIV replication has been well established in tissue culture models of infection (Sher et al., 1992 Immun. Rev. 127, 183), suggesting that the mutual induction of HIV replication and TNF-α replication may create positive feedback *in vivo*. However, evidence for the presence of such positive feedback in infected patients is not abundant. TNF-α levels are found to be elevated in some, but not all patients tested. Children with AIDS who were given zidovudine had reduced levels of TNF-α compared to those not given zidovudine (Cremoni et al., 1993 AIDS 7, 128). This correlation lends support to the hypothesis that reduced viral replication is physiologically linked to TNF-α levels. Furthermore, recently it has been shown that the polyclonal B cell activation associated with HIV infection is due to membrane-bound TNF-α. Thus, levels of secreted TNF-α may not accurately reflect the contribution of this cytokine to AIDS pathogenesis.

Chronic elevation of TNF-α has been shown to shown to result in cachexia (Tracey et al., 1992 Am. J. Trop. Med. Hyg. 47, 2-7), increased autoimmune disease (Jacob, 1992 supra), lethargy, and immune suppression in animal models (Aderka et al., 1992 Isr. J. Med. Sci. 28, 126-130). The cachexia associated with AIDS may be associated with chronically elevated TNF-α frequently observed in AIDS patients. Similarly, TNF-α can stimulate the proliferation of spindle cells isolated from Kaposi's sarcoma lesions of AIDS patients (Barillari et al., 1992 J Immunol 149, 3727).

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves the specified sites in TNF-α mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

•Septic shock.

Exogenous delivery of ribozymes to macrophages can be achieved by intraperitoneal or intravenous injections. Ribozymes will be delivered by incorporation into liposomes or by complexing with cationic lipids.

•Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Psoriasis

The psoriatic plaque is a particularly good candidate for ribozyme or vector delivery. The stratum corneum of the plaque is thinned, providing access to the proliferating keratinocytes. T-cells and dermal dendrocytes can be efficiently targeted by trans-epidermal diffusion .

Organ culture systems for biopsy specimens of psoriatic and normal skin are described in current literature (Nickoloff et al., 1993 Supra). Primary human keratinocytes are easily obtained and will be grown into epidermal sheets in tissue culture. In addition to these tissue culture models, the flaky skin mouse develops psoriatic skin in response to UV light. This model would allow demonstration of animal efficacy for ribozyme treatments of psoriasis.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus

vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave TNF-α mRNA and thereby TNF-α activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits TNF-α function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for TNF-α function and for each of the suggested disease targets exist and can be used to optimize activity.

Example 5: p210$^{bcr-abl}$

Chronic myelogenous leukemia exhibits a characteristic disease course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype (i.e., the blast crisis stage of the disease). CML is an unstable disease which ultimately progresses to a terminal stage which resembles acute leukemia. This lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents such as hydroxyurea or busulfan can reduce the leukemic burden but do not impact the life expectancy of the patient (e.g. approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients which survive BMT, disease recurrence remains a major obstacle (Apperley et al., 1988 Br. J. Haematol. 69, 239).

The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-25% of all cases of acute lymphoblastic leukemia [(ALL); Fourth International Workshop on Chromosomes in Leukemia 1982, Cancer Genet. Cytogenet. 11, 316]. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express *bcr-abl* fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2 junction) from the major breakpoint cluster region of the *bcr* gene is spliced

to exon 2 of the *abl* gene. Heisterkamp et al., 1985 Nature 315, 758; Shtivelman et al., 1987, Blood 69, 971). In the remaining cases of Ph-positive ALL, the first exon of the *bcr* gene is spliced to exon 2 of the *abl* gene (Hooberman et al., 1989 Proc. Nat. Acad. Sci. USA 86, 4259; Heisterkamp et al., 1988 Nucleic Acids Res. 16, 10069).

The b3-a2 and b2-a2 fusion mRNAs encode 210 kd bcr-abl fusion proteins which exhibit oncogenic activity (Daley et al., 1990 Science 247, 824; Heisterkamp et al., 1990 Nature 344, 251). The importance of the bcr-abl fusion protein (p210$^{bcr\text{-}abl}$) in the evolution and maintenance of the leukemic phenotype in human disease has been demonstrated using antisense oligonucleotide inhibition of p210$^{bcr\text{-}abl}$ expression. These inhibitory molecules have been shown to inhibit the in vitro proliferation of leukemic cells in bone marrow from CML patients. Szczylik et al., 1991 Science 253, 562).

Reddy, U.S. Patent 5,246,921 (hereby incorporated by reference herein) describes use of ribozymes as therapeutic agents for leukemias, such as chronic myelogenous leukemia (CML) by targeting the specific junction region of *bcr-abl* fusion transcripts. It indicates causing cleavage by a ribozyme at or near the breakpoint of such a hybrid chromosome, specifically it includes cleavage at the sequence GUX, where X is A, U or G. The one example presented is to cleave the sequence 5' AGC AG AGUU (cleavage site) CAA AAGCCCU-3'.

Scanlon WO 91/18625, WO 91/18624, and WO 91/18913 and Snyder et al., WO93/03141 and WO94/13793 describe a ribozyme effective to cleave oncogenic variants of H-*ras* RNA. This ribozyme is said to inhibit H-*ras* expression in response to external stimuli.

The invention features use of ribozymes to inhibit the development or expression of a transformed phenotype in man and other animals by modulating expression of a gene that contributes to the expression of CML. Cleavage of targeted mRNAs expressed in pre-neoplastic and transformed cells elicits inhibition of the transformed state.

The invention can be used to treat cancer or pre-neoplastic conditions. Two preferred administration protocols can be used, either in vivo administration to reduce the tumor burden, or ex vivo treatment to

eradicate transformed cells from tissues such as bone marrow prior to reimplantation.

This inv ntion features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of CML. The mRNA targets are present in the 425 nucleotides surrounding the fusion sites of the *bcr* and *abl* sequences in the b2-a2 and b3-a2 recombinant mRNAs. Other sequences in the 5' portion of the *bcr* mRNA or the 3' portion of the *abl* mRNA may also be targeted for ribozyme cleavage. Cleavage at any of these sites in the fusion mRNA molecules will result in inhibition of translation of the fusion protein in treated cells.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of CML. Such enzymatic RNA molecules can be delivered exogenously or endogenously to afflicted cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The smallest ribozyme delivered for any type of treatment reported to date (by Rossi et al., 1992 supra) is an in vitro transcript having a length of 142 nucleotides. Synthesis of ribozymes greater than 100 nucleotides in length is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression vectors is primarily feasible using only ex vivo treatments. This limits the utility of this approach. In this invention, an alternative approach uses smaller ribozyme motifs and exogenous delivery. The simple structure of these molecules also increases the ability of the ribozyme to invade targeted regions of the mRNA structure. Thus, unlike the situation when the hammerhead structure is included within longer transcripts, there are no non-ribozyme flanking sequences to interfere with correct folding of the ribozyme structure, as well as complementary binding of the ribozyme to the mRNA target.

The enzymatic RNA molecules of this invention can be used to treat human CML or precancerous conditions. Affected animals can be treated at the time of cancer detection or in a prophylactic manner. This timing of treatment will reduce the number of affected cells and disable cellular

replication. This is possible because the ribozymes are designed to disable those structures required for successful cellular proliferation.

Ribozymes of this invention block to some extent p210*bcr-abl* expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to tissues in animal models of CML. Ribozyme cleavage of *bcr/abl* mRNA in these systems may prevent or alleviate disease symptoms or conditions.

The sequence of human *bcr/abl* mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Table 29 (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

The sequences of the chemically synthesized ribozymes most useful in this study are shown in Table 30. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Table 30 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 30 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *bcr-abl* mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance as described above. These ribozymes cleave *bcr-abl* target sequences *in vitro.*

The ribozymes are tested for function *in vivo* by exogenous delivery to cells expressing *bcr-abl*. Ribozymes are delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. Expression of *bcr-abl* is monitored by ELISA, by indirect immunofluoresence, and/or by FACS analysis. Levels of

bcr-abl mRNA are assessed by Northern analysis, RNase protection, by primer extension analysis or by quantitative RT-PCR techniques. Ribozymes that block the induction of p210*bcr-abl*) protein and mRNA by more than 20% are identified.

Example 6: RSV

This invention relates to the use of ribozymes as inhibitors of respiratory syncytial virus (RSV) production, and in particular, the inhibition of RSV replication.

RSV is a member of the virus family paramyxoviridae and is classified under the genus *Pneumovirus* (for a review see McIntosh and Chanock, 1990 in Virology ed. B.N. Fields, pp. 1045, Raven Press Ltd. NY). The infectious virus particle is composed of a nucleocapsid enclosed within an envelope. The nucleocapsid is composed of a linear negative single-stranded non-segmented RNA associated with repeating subunits of capsid proteins to form a compact structure and thereby protect the RNA from nuclease degradation. The entire nucleocapsid is enclosed by the envelope. The size of the virus particle ranges from 150 - 300 nm in diameter. The complete life cycle of RSV takes place in the cytoplasm of infected cells and the nucleocapsid never reaches the nuclear compartment (Hall, 1990 in Principles and Practice of Infectious Diseases ed. Mandell et al., Churchill Livingstone, NY).

The RSV genome encodes ten viral proteins essential for viral production. RSV protein products include two structural glycoproteins (G and F) found in the envelope spikes, two matrix proteins [M and M2 (22K)] found in the inner membrane, three proteins localized in the nucleocapsid (N, P and L), one protein that is present on the surface of the infected cell (SH), and two nonstructural proteins [NS1 (1C) and NS2 (1B)] found only in the infected cell. The mRNAs for the 10 RSV proteins have similar 5' and 3' ends. UV-inactivation studies suggest that a single promoter is used with multiple transcription initiation sites (Barik *et al.*, 1992 J. Virol. 66, 6813). The order of transcription corresponding to the protein assignment on the genomic RNA is 1C, 1B, N, P, M, SH, G, F, 22K and L genes (Huang *et al.*, 1985 Virus Res. 2, 157) and transcript abundance corresponds to the order of gene assignment (for example the 1C and 1B mRNAs are much more abundant than the L mRNA. Synthesis of viral message begins

immediately after RSV infection of cells and reaches a maximum at 14 hours post-infection (McIntosh and Chanock, *supra*).

There are two antigenic subgroups of RSV, A and B, which can circulate simultaneously in the community in varying proportions in different years (McIntosh and Chanock, *supra*). Subgroup A usually predominates. Within the two subgroups there are numerous strains. By the limited sequence analysis available it seems that homology at the nucleotide level is more complete within than between subgroups, although sequence divergence has been noted within subgroups as well. Antigenic determinates result primarily from both surface glycoproteins, F and G. For F, at least half of the neutralization epitopes have been stably maintained over a period of 30 years. For G however, A and B subgroups may be related antigenically by as little as a few percent. On the nucleotide level, however, the majority of the divergence in the coding region of G is found in the sequence for the extracellular domain (Johnson et al., 1987, *Proc. Natl. Acad. Sci.* USA 84, 5625).

Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract illness during infancy and childhood (Hall, *supra*) and as such is associated with an estimated 90,000 hospitalizations and 4500 deaths in the United States alone (Update: respiratory syncytial virus activity - United States, 1993, Mmwr Morb Mortal Wkly Rep, 42, 971). Infection with RSV generally outranks all other microbial agents leading to both pneumonia and bronchitis. While primarily affecting children under two years of age, immunity is not complete and reinfection of older children and adults, especially hospital care givers (McIntosh and Chanock, *supra*), is not uncommon. Immunocompromised patients are severely affected and RSV infection is a major complication for patients undergoing bone marrow transplantation .

Uneventful RSV respiratory disease resembles a common cold and recovery is in 7 to 12 days. Initial symptoms (rhinorrhea, nasal congestion, slight fever, etc.) are followed in 1 to 3 days by lower respiratory tract signs of infection that include a cough and wheezing. In severe cases, these mild symptoms quickly progress to tachypnea, cyanosis, and listlessness and hospitalization is required. In infants with underlying cardiac or respiratory disease, the progression of symptoms is especially rapid and can lead to respiratory failure by the second or third day of illness. With

modem intensive care however, overall mortality is usually less than 5% of hospitalized patients (McIntosh and Chanock, *supra*).

At present, neither an efficient vaccine nor a specific antiviral agent is available. An immune response to the viral surface glycoproteins can provide resistance to RSV in a number of experimental animals, and a subunit vaccine has been shown to be effective for up to 6 months in children previously hospitalized with an RSV infection (Tristam *et al.*, 1993, J. Infect. Dis. 167, 191). An attenuated bovine RSV vaccine has also been shown to be effective in calves for a similar length of time (Kubota *et al.*, 1992 J. Vet. Med. Sci. 54, 957). Previously however, a formalin-inactivated RSV vaccine was implicated in greater frequency of severe disease in subsequent natural infections with RSV (Connors *et al.*, 1992 J. Virol. 66, 7444).

The current treatment for RSV infection requiring hospitalization is the use of aerosolized ribavirin, a guanosine analog [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY.]. Ribavirin therapy is associated with a decrease in the severity of the symptoms, improved arterial oxygen and a decrease in the amount of viral shedding at the end of the treatment period. It is not certain, however, whether ribavirin therapy actually shortens the patients' hospital stay or diminishes the need for supportive therapies (McIntosh and Chanock, *supra*). The benefits of ribavirin therapy are especially clear for high risk infants, those with the most serious symptoms or for patients with underlying bronchopulmonary or cardiac disease. Inhibition of the viral polymerase complex is supported as the main mechanism for inhibition of RSV by ribavirin, since viral but not cellular polypeptide synthesis is inhibited by ribavirin in RSV-infected cells (Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY]. Since ribavirin is at least partially effective against RSV infection when delivered by aerosolization, it can be assumed that the target cells are at or near the epithelial surface. In this regard, RSV antigen had not spread any deeper than the superficial layers of the respiratory epithelium in autopsy studies of fatal pneumonia (McIntosh and Chanock, *supra*).

Jennings *et al.*, WO 94/13688 indicates that targets for specific types of ribozymes include respiratory syncytical virus.

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting production of respiratory syncytial virus (RSV). Such ribozymes can be used in a method for treatment of diseases caused by these related viruses in man and other animals. The invention also features cleavage of the genomic RNA and mRNA of these viruses by use of ribozymes. In particular, the ribozyme molecules described are targeted to the *NS1 (1C), NS2 (1B)* and *N* viral genes. These genes are known in the art (for a review see McIntosh and Chanock, 1990 *supra*).

Ribozymes that cleave the specified sites in RSV mRNAs represent a novel therapeutic approach to respiratory disorders. Applicant indicates that ribozymes are able to inhibit the activity of RSV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these sites in RSV mRNAs encoding 1C, 1B and N proteins may be readily designed and are within the invention. Also, those of ordinary skill in the art, will find that it is clear from the examples described that ribozymes cleaving other mRNAs encoded by RSV (*P, M, SH, G, F, 22K* and *L*) and the genomic RNA may be readily designed and are within the invention.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 31, 33, 35, 37 and 38. Examples of such ribozymes are shown in Tables 32, 34, 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Ribozymes of this invention block to some extent RSV production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of respiratory disorders. Ribozyme cleavage of RSV encoded mRNAs or the genomic RNA in these systems may alleviat disease symptoms.

While all ten RSV encoded proteins (1C, 1B, N, P, M, SH, 22K, F, G, and L) are essential for viral life cycle and are all potential targets for ribozyme cleavage, certain proteins (mRNAs) are more favorable for ribozyme targeting than the others. For example RSV encoded proteins 1C, 1B, SH and 22K are not found in other members of the family paramyxoviridae and appear to be unique to RSV. In contrast the ectodomain of the G protein and the signal sequence of the F protein show significant sequence divergence at the nucleotide level among various RSV sub-groups (Johnson *et al.*, 1987 *supra*). RSV proteins 1C, 1B and N are highly conserved among various subtypes at both the nucleotide and amino acid levels. Also, 1C, 1B and N are the most abundant of all RSV proteins.

The sequence of human RSV mRNAs encoding 1C, 1B and N proteins are screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 31, 33, 34, 37 and 38 (All sequences are 5' to 3' in the tables.) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G_5 and a U for A_{14} (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Hairpin ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant

groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-o-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 32, 34, 36, 37 and 38. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 32 and 34(5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 37 and 38 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 32, 34, 36, 37 and 38 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against RSV encoded mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* is evaluated.

Numerous common cell lines can be infected with RSV for experimental purposes. These include *HeLa*, *Vero* and several primary epithelial cell lines. A cotton rat animal model of experimental human RSV infection is also available, and the bovine RSV is quite homologous to the human viruses. Rapid clinical diagnosis is through the use of kits designed for the immunofluorescence staining of RSV-infected cells or an ELISA assay, both of which are adaptable for experimental study. RSV encoded mRNA levels will be assessed by Northern analysis, RNAse protection, primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of RSV activity and/or 1C, 1B and N protein encoding mRNAs by more than 90% will be identified.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., PCT WO93/23569. The details will not b repeated here, but include altering the length of the ribozyme binding arms or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.,* Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Jennings *et al.*, WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., PCT WO94/02595, incorporated by reference herein, describes the general methods for delivery of enzymatic RNA molecules . Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., supra and Draper, et al., supra which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given

pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisziewicz et al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral, or alpha virus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves target RNA is inserted into a plasmid DNA vector, a retrovirus DNA viral vector, an adenovirus DNA viral vector or an adeno-associated virus vector or alpha virus vector. These and other vectors have been used to transfer genes to live animals (for a review see Friedman, 1989 Science 244, 1275-1281; Roemer and Friedman, 1992 Eur. J. Biochem. 208, 211-225) and leads to transient or stable gene expression. The vectors are delivered as recombinant viral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment, *e.g.*, through the use of a catheter, stent or infusion pump.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By

using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with ICAM-1, relA, TNF-α, p210, $^{bcr\text{-}abl}$ or RSV related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., ICAM-1, rel A, TNF∝, p210$^{bcr\text{-}abl}$ or RSV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will

decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

II. Chemical Synthesis Of Ribozymes

There follows the chemical synthesis, deprotection, and purification of RNA, enzymatic RNA or modified RNA molecules in greater than milligram quantities with high biological activity. Applicant has determined that the synthesis of enzymatically active RNA in high yield and quantity is dependent upon certain critical steps used during its preparation. Specifically, it is important that the RNA phosphoramidites are coupled efficiently in terms of both yield and time, that correct exocyclic amino protecting groups be used, that the appropriate conditions for the removal of the exocyclic amino protecting groups and the alkylsilyl protecting groups on the 2'-hydroxyl are used, and that the correct work-up and purification procedure of the resulting ribozyme be used.

To obtain a correct synthesis in terms of yield and biological activity of a large RNA molecule (*i.e.*, about 30 to 40 nucleotide bases), the protection of the amino functions of the bases requires either amide or substituted amide protecting groups, which must be, on the one hand, stable enough to survive the conditions of synthesis, and on the other hand, removable at the end of the synthesis. These requirements are met by the amide protecting groups shown in Figure 8, in particular, benzoyl for adenosine, isobutyryl or benzoyl for cytidine, and isobutyryl for guanosine, which may be removed at the end of the synthesis by incubating the RNA in NH_3/EtOH (ethanolic ammonia) for 20 h at 65 °C. In the case of the phenoxyacetyl type protecting groups shown in Figure 8 on guanosine and adenosine and acetyl protecting groups on cytidine, an incubation in ethanolic ammonia for 4 h at 65 °C is used to obtain complete removal of these protecting groups. Removal of the alkylsilyl 2'-hydroxyl protecting groups can be accomplished using a tetrahydrofuran solution of TBAF at room temperature for 8-24 h.

The most quantitative procedure for recovering the fully deprotected RNA molecule is by either ethanol precipitation, or an anion exchange cartridge desalting, as described in Scaringe *et al. Nucleic Acids Res.* 1990, *18*, 5433-5341. The purification of the long RNA sequences may be

accomplished by a two-step chromatographic procedur in which the molecule is first purified on a reverse phase column with either the trityl group at the 5' position on or off. This purification is accomplished using an acetonitrile gradient with triethylammonium or bicarbonate salts as the aqueous phase. In the case of the trityl on purification, the trityl group may be removed by the addition of an acid and drying of the partially purified RNA molecule. The final purification is carried out on an anion exchange column, using alkali metal perchlorate salt gradients to elute the fully purified RNA molecule as the appropriate metal salts, *e.g.* Na^+, Li^+ *etc.* A final de-salting step on a small reverse-phase cartridge completes the purification procedure. Applicant has found that such a procedure not only fails to adversely affect activity of a ribozyme, but may improve its activity to cleave target RNA molecules.

Applicant has also determined that significant (see Tables 39-41) improvements in the yield of desired full length product (FLP) can be obtained by:

1. Using 5-*S*-alkyltetrazole at a delivered or effective concentration of 0.25-0.5 M or 0.15-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. (By delivered is meant that the actual amount of chemical in the reaction mix is known. This is possible for large scale synthesis since the reaction vessel is of size sufficient to allow such manipulations. The term effective means that available amount of chemical actually provided to the reaction mixture that is able to react with the other reagents present in the mixture. Those skilled in the art will recognize the meaning of these terms from the examples provided herein.) The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m. Alkyl, as used herein, refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to

7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above. Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

2. Using 5-*S*-alkyltetrazole at an effective, or final, concentration of 0.1-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m.

3. Using alkylamine (MA, wher alkyl is preferably methyl, ethyl, propyl or butyl) or NH_4OH/alkylamine (AMA, with the same preferred alkyl groups as noted for MA) @ 65 °C for 10-15 m to remove the exocyclic

amino protecting groups (*vs* 4-20 h @ 55-65 °C using NH_4OH/EtOH or NH_3/EtOH, *vide supra*). Other alkylamines, *e.g.* ethylamine, propylamine, butylamine *etc.* may also be used.

4. Using anhydrous triethylamine•hydrogen fluoride (aHF•TEA) @ 65 °C for 0.5-1.5 h to remove the 2'-hydroxyl alkylsilyl protecting group (*vs* 8 - 24 h using TBAF, *vide supra* or TEA•3HF for 24 h (Gasparutto *et al. Nucleic Acids Res.* **1992**, 20, 5159-5166). Other alkylamine•HF complexes may also be used, *e.g.* trimethylamine or diisopropylethylamine.

5. The use of anion-exchange resins to purify and/or analyze the fully deprotected RNA. These resins include, but are not limited to, quartenary or tertiary amino derivatized stationary phases such as silica or polystyrene. Specific examples include Dionex-NA100®, Mono-Q®, Poros-Q®.

Thus, the invention features an improved method for the coupling of RNA phosphoramidites; for the removal of amide or substituted amide protecting groups; and for the removal of 2'-hydroxyl alkylsilyl protecting groups. Such methods enhance the production of RNA or analogs of the type described above (*e.g.,* with substituted 2'-groups), and allow efficient synthesis of large amounts of such RNA. Such RNA may also have enzymatic activity and be purified without loss of that activity. While specific examples are given herein, those in the art will recognize that equivalent chemical reactions can be performed with the alternative chemicals noted above, which can be optimized and selected by routine experimentation.

In another aspect, the invention features an improved method for the purification or analysis of RNA or enzymatic RNA molecules (*e.g.* 28-70 nucleotides in length) by passing said RNA or enzymatic RNA molecule over an HPLC, *e.g.,* reverse phase and/or an anion exchange chromatography column. The method of purification improves the catalytic activity of enzymatic RNAs over the gel purification method (see Figure 10).

Draper et al., PCT WO93/23569, incorporated by reference herein, disclosed reverse phase HPLC purification. The purification of long RNA molecules may be accomplished using anion exchange chromatography, particularly in conjunction with alkali perchlorate salts. This system may be used to purify very long RNA molecules. In particular, it is advantageous to

use a Dionex NucleoPak 100© or a Pharmacia Mono Q® anion exchange column for th purification of RNA by the anion exchange method. This anion exchange purification may be used following a reverse-phase purification or prior to reverse phase purification. This method results in the formation of a sodium salt of the ribozyme during the chromatography. Replacement of the sodium alkali earth salt by other metal salts, *e.g.*, lithium, magnesium or calcium perchlorate, yields the corresponding salt of the RNA molecule during the purification.

In the case of the 2-step purification procedure, in which the first step is a reverse phase purification followed by an anion exchange step, the reverse phase purification is best accomplished using polymeric, *e.g.* polystyrene based, reverse-phase media, using either a 5'-trityl-on or 5'-trityl-off method. Either molecule may be recovered using this reverse-phase method, and then, once detritylated, the two fractions may be pooled and then submitted to an anion exchange purification step as described above.

The method includes passing the enzymatically active RNA molecule over a reverse phase HPLC column; the enzymatically active RNA molecule is produced in a synthetic chemical method and not by an enzymatic process; and the enzymatic RNA molecule is partially blocked, and the partially blocked enzymatically active RNA molecule is passed over a reverse phase HPLC column to separate it from other RNA molecules.

In more preferred embodiments, the enzymatically active RNA molecule, after passage over the reverse phase HPLC column, is deprotected and passed over a second reverse phase HPLC column (which may be the same as the reverse phase HPLC column), to remove the enzymatic RNA molecule from other components. In addition, the column is a silica or organic polymer-based C4, C8 or C18 column having a porosity of at least 125 Å, preferably 300 Å, and a particle size of at least 2 μm, preferably 5 μm.

<u>Activation</u>

The synthesis of RNA molecules may be accomplished chemically or enzymatically. In the case of chemical synthesis the use of tetrazole as an activator of RNA phosphoramidites is known (Usman *et al.* *J. Am. Chem.*

Soc. **1987**, 109, 7845-7854). In this, and subsequent reports, a 0.5 M solution of tetrazole is allowed to react with the RNA phosphoramidite and couple with the polymer bound 5'-hydroxyl group for 10 m. Applicant has determined that using 0.25-0.5 M solutions of 5-*S*-alkyltetrazoles for only 5 min gives equivalent or better results. The following exemplifies the procedure.

Example 7: Synthesis of RNA and Ribozymes Using 5-*S*-Alkyltetrazoles as Activating Agent

The method of synthesis used follows the general procedure for RNA synthesis as described in Usman et al., 1987*supra* and in Scaringe et al., *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The major difference used was the activating agent, 5-*S*-ethyl or -methyltetrazole @ 0.25 M concentration for 5 min.

All small scale syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 µmol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 m coupling step for 2'-*O*-methylated RNA. A 6.5-fold excess (162.5 µL of 0.1 M = 32.5 µmol) of phosphoramidite and a 40-fold excess of *S*-ethyl tetrazole (400 µL of 0.25 M = 100 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I_2, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

All large scale syntheses were conducted on a modified (eight amidite port capacity) 390Z (ABI) synthesizer using a 25 µmol scale protocol with a 5-15 min coupling step for alkylsilyl protected RNA and 7.5 m coupling step for 2'-*O*-methylated RNA. A six-fold excess (1.5 mL of 0.1 M = 150 µmol) of phosphoramidite and a forty-five-fold excess of *S*-ethyl tetrazole (4.5 mL of

0.25 M = 1125 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 390Z, determined by colorimetric quantitation of the trityl fractions, was 95.0-96.7%. Oligonucleotide synthesis reagents for the 390Z: Detritylation solution was 2% DCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I_2, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25-0.5 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

Deprotection

The first step of the deprotection of RNA molecules may be accomplished by removal of the exocyclic amino protecting groups with either NH_4OH/EtOH:3/1 (Usman *et al. J. Am. Chem. Soc.* 1987, *109*, 7845-7854) or NH_3/EtOH (Scaringe *et al. Nucleic Acids Res.* 1990, *18*, 5433-5341) for ~20 h @ 55-65 °C. Applicant has determined that the use of methylamine or NH_4OH/methylamine for 10-15 min @ 55-65 °C gives equivalent or better results. The following exemplifies the procedure.

Example 8: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using Methylamine (MA) or NH_4OH/Methylamine (AMA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of methylamine (MA) or NH_4OH/methylamine (AMA) @ 55-65 °C for 5-15 min to remove the exocyclic amino protecting groups. The polymer-bound oligoribonucleotide was transferred from the synthesis column to a 4 mL glass screw top vial. NH_4OH and aqueous methylamine were pre-mixed in equal volumes. 4 mL of the resulting reagent was added to the vial, equilibrated for 5 m at RT and then heated at 55 or 65 °C for 5-15 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed with 1.0 mL of EtOH:MeCN:H_2O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing th oligoribonucleotide, were dried to a white powder. The same procedure was followed for the aqueous methylamine reagent.

Table 40 is a summary of the results obtained using the improvements outlined in this application for base deprotection.

The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al.* *J. Am. Chem. Soc.* 1987, *109*, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results. The following exemplifies this procedure.

Example 9: RNA and Ribozyme Deprotection of 2'-Hydroxyl Alkylsilyl Protecting Groups Using Anhydrous TEA•HF

To remove the alkylsilyl protecting groups, the ammonia-deprotected oligoribonucleotide was resuspended in 250 µL of 1.4 M anhydrous HF solution (1.5 mL *N*-methylpyrrolidine, 750 µL TEA and 1.0 mL TEA•3HF) and heated to 65 °C for 1.5 h. 9 mL of 50 mM TEAB was added to quench the reaction. The resulting solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) prewashed with 10 mL of 50 mM TEAB. After washing the cartridge with 10 mL of 50 mM TEAB, the RNA was eluted with 10 mL of 2 M TEAB and dried down to a white powder.

Table 41 is a summary of the results obtained using the improvements outlined in this application for alkylsilyl deprotection.

Example 10: HPLC Purification, Anion Exchange column

For a small scale synthesis, the crude material was diluted to 5 mL with diethylpyrocarbonate treated water. The sample was injected onto either a Pharmacia Mono Q® 16/10 or Dionex NucleoPac® column with 100% buffer A (10 mM $NaClO_4$). A gradient from 180-210 mM $NaClO_4$ at a rate of 0.85 mM/void volume for a Pharmacia Mono Q® anion-exchange column or 100-150 mM $NaClO_4$ at a rate of 1.7 mM/void volume for a Dionex NucleoPac® anion-exchange column was used to elute the RNA. Fractions were analyzed by a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing full length product at ≥80% by peak area were pooled.

For a trityl-off large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 10 mM sodium perchlorate buffer. The oligonucleotide was eluted from the column with

300 mM sodium perchlorate. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material in the synthesis. The eluent was diluted four fold in sterile H_2O to lower the salt concentration and applied to a Pharmacia Mono Q® 16/10 column. A gradient from 10-185 mM sodium perchlorate was run over 4 column volumes to elute shorter sequences, the full length product was then eluted in a gradient from 185-214 mM sodium perchlorate in 30 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing over 85% full length material were pooled. The pool was applied to a Pharmacia RPC® column for desalting.

For a trityl-on large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 20 mM NH_4CO_3H/10% CH_3CN buffer. The oligonucleotide was eluted from the column with 1.5 M NH_4CO_3H/10% acetonitrile. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material present in the synthesis. The oligonucleotide was then applied to a Pharmacia Resource RPC column. A gradient from 20-55% B (20 mM NH_4CO_3H/25% CH_3CN, buffer A = 20 mM NH_4CO_3H/10% CH_3CN) was run over 35 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing over 60% full length material were pooled. The pooled fractions were then submitted to manual detritylation with 80% acetic acid, dried down immediately, resuspended in sterile H_2O, dried down and resuspended in H_2O again. This material was analyzed on a HP 1090-HPLC with a Dionex NucleoPac® column. Th material was purified by anion exchange chromatography as in the trityl-off scheme (*vide supra*).

Example 11 Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 µM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations wer ~ 1 nM. Total reaction volumes were 50 µL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM $MgCl_2$. Reactions were

initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 µL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each aliquot was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 12: One pot deprotection of RNA

Applicant has shown that aqueous methyl amine is an efficient reagent to deprotect bases in an RNA molecule. However, in a time consuming step (2-24 hrs), the RNA sample needs to be dried completely prior to the deprotection of the sugar 2'-hydroxyl groups. Additionally, deprotection of RNA synthesized on a large scale (e.g., 100 µmol) becomes challenging since the volume of solid support used is quite large. In an attempt to minimize the time required for deprotection and to simplify the process of deprotection of RNA synthesized on a large scale, applicant describes a one pot deprotection protocol (Fig. 12). According to this protocol, anhydrous methylamine is used in place of aqueous methyl amine. Base deprotection is carried out at 65 °C for 15 min and the reaction is allowed to cool for 10 min. Deprotection of 2'-hydroxyl groups is then carried out in the same container for 90 min in a TEA•3HF reagent. The reaction is quenched with 16 mM TEAB solution.

Referring to Fig. 13, hammerhead ribozyme targeted to site B is synthesized using RNA phosphoramadite chemistry and deprotected using either a two pot or a one pot protocol. Profiles of these ribozymes on an HPLC column are compared. The figure shows that RNAs deprotected by either the one pot or the two pot protocols yield similar full-length product profiles. Applicant has shown that using a one pot deprotection protocol, time required for RNA deprotection can be reduced considerably without compromising the quality or the yield of full length RNA.

Referring to Fig. 14, hammerhead ribozymes targeted to site B (from Fig. 13) are tested for their ability to cleave RNA. As shown in the figure 14, ribozymes that are deprotected using one pot protocol have catalytic activity comparable to ribozymes that are deprotected using a two pot protocol.

Example 12a: Improved protocol for the synthesis of phosphorothioate containing RNA and ribozymes using 5-S-Alkyltetrazoles as Activating Agent

The two sulfurizing reagents that have been used to synthesize ribophosphorothioates are tetraethylthiuram disulfide (TETD; Vu and Hirschbein, 1991 *Tetrahedron Letter* 31, 3005), and 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent; Vu and Hirschbein, 1991 *supra*). TETD requires long sulfurization times (600 seconds for DNA and 3600 seconds for RNA). It has recently been shown that for sulfurization of DNA oligonucleotides, Beaucage reagent is more efficient than TETD (Wyrzykiewicz and Ravikumar, 1994 *Bioorganic Med. Chem.* 4, 1519). Beaucage reagent has also been used to synthesize phosphorothioate oligonucleotides containing 2'-deoxy-2'-fluoro modifications wherein the wait time is 10 min (Kawasaki et al., 1992 *J. Med. Chem*).

The method of synthesis used follows the procedure for RNA synthesis as described herein and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The sulfurization step for RNA described in the literature is a 8 second delivery and 10 min wait steps (Beaucage and Iyer, 1991 *Tetrahedron* 49, 6123). These conditions produced about 95% sulfurization as measured by HPLC analysis (Morvan et al., 1990 *Tetrahedron Letter* 31, 7149). This 5% contaminating oxidation could arise from the presence of oxygen dissolved in solvents and/or slow release of traces of iodine adsorbed on the inner surface of delivery lines during previous synthesis.

A major improvement is the use of an activating agent, 5-*S*-ethyltetrazole or 5-*S*-methyltetrazole at a concentration of 0.25 M for 5 min. Additionally, for those linkages which are phosporothioate, the iodine solution is replaced with a 0.05 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) in acetonitrile. The delivery time for the sulfurization step is reduced to 5 seconds and the wait time is reduced to 300 seconds.

RNA synthesis is conducted on a 394 (ABI) synthesizer using a modified 2.5 μmol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 min coupling step for 2'-*O*-methylated RNA. A 6.5-fold excess (162.5 μL of 0.1 M = 32.5 μmol) of phosphoramidite

and a 40-fold excess of *S*--ethyl tetrazole (400 μL of 0.25 M = 100 μmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 synthesizer, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394 synthesizer: detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I_2, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems. Sulfurizing reagent was obtained from Glen Research.

Average sulfurization efficiency (ASE) is determined using the formula: $ASE = (PS/Total)^{1/n-1}$

where, PS = integrated ^{31}P NMR values of the P=S diester

Total = integration value of all peaks

n = length of oligo

Referring to tables 42 and 43, effects of varying the delivery and the wait time for sulfurization with Beaucage's reagent is described. These data suggest that 5 second wait time and 300 second delivery time is the condition under which ASE is maximum.

Using the above conditions a 36 mer hammerhead ribozyme is synthesized which is targeted to site C. The ribozyme is synthesized to contain phosphorothioate linkages at four positions towards the 5' end. RNA cleavage activity of this ribozyme is shown in Fig. 16. Activity of the phosphorothioate ribozyme is comparable to the activity of a ribozyme lacking any phosphorothioate linkages.

Example 13: Protocol for the synthesis of 2'-N-phtalimido-nucleoside phosphoramidite

The 2'-amino group of a 2'-deoxy-2'-amino nucleoside is normally protected with N-(9-flourenylmethoxycarbonyl) (Fmoc; Imazawa and Eckstein, 1979 *supra*; Pieken et al., 1991 *Science* 253, 314). This protecting group is not stable in CH_3CN solution or even in dry form during

prolonged storage at -20 °C. These problems need to be overcome in order to achieve large scale synthesis of RNA.

Applicant describes the use of alternative protecting groups for the 2'-amino group of 2'-deoxy-2'-amino nucleoside. Referring to Figure 17, phosphoramidite **17** was synthesized starting from 2'-deoxy-2'-aminonucleoside (**12**) using transient protection with Markevich reagent (Markiewicz *J. Chem. Res.* 1979, S, 24). An intermediate **13** was obtained in 50% yield, however subsequent introduction of N-phtaloyl (Pht) group by Nefken's method (Nefkens, 1960 *Nature* 185, 306), desilylation (**15**), dimethoxytrytilation (**16**) and phosphitylation led to phosphoramidite **17**. Since overall yield of this multi-step procedure was low (20%) applicant investigated some alternative approaches, concentrating on selective introduction of N-phtaloyl group without acylation of 5' and 3' hydroxyls.

When 2'-deoxy-2'-amino-nucleoside was reacted with 1.05 equivalents of Nefkens reagent in DMF overnight with subsequent treatment with Et_3N (1 hour) only 10-15% of N and 5'(3')-bis-phtaloyl derivatives were formed with the major component being N-Pht-derivative **15**. The N,O-bis by-products could be selectively and quantitively converted to N-Pht derivative **15** by treatment of crude reaction mixture with cat. KCN/MeOH.

A convenient "one-pot" procedure for the synthesis of key intermediate **16** involves selective N-phthaloylation with subsequent dimethoxytrytilation by DMTCl/Et_3N and resulting in the preparation of DMT derivative **16** in 85% overall yield as follows. Standard phosphytilation of **16** produced phosphoramidite **17** in 87% yield. One gram of 2'-amino nucleoside, for example 2'-amino uridine (US Biochemicals® part # 77140) was co-evaporated twice from dry dimethyl formamide (Dmf) and dried in vacuo overnight. 50 mls of Aldrich sure-seal Dmf was added to the dry 2'-amino uridine via syringe and the mixture was stirred for 10 minutes to produce a clear solution. 1.0 grams (1.05 eq.) of N-carbethoxyphthalimide (Nefken's reagent, 98% Jannsen Chimica) was added and the solution was stirred overnight. Thin layer chromatography (TLC) showed 90% conversion to a faster moving products (10% ETOH in $CHCl_3$) and 57 µl of TEA (0.1 eq.) was added to effect closure of the phthalimide ring. After 1 hour an additional 855 µl (1.5 eq.) of TEA was added followed by the addition of 1.53 grams (1.1 eq.) of DMT-Cl

(Lancast r Synthesis®, 98%). The reaction mixture was left to stir overnight and quenched with ETOH after TLC showed greater than 90% desired product. Dmf was removed under vacuum and the mixture was washed with sodium bicarbonate solution (5% aq., 500 mls) and extracted with ethyl acetate (2x 200 mls). A 25mm x 300mm flash column (75 grams Merck flash silica) was used for purification. Compound eluted at 80 to 85% ethyl acetate in hexanes (yield: 80% purity: >95% by ^{1}HNMR). Phosphoramidites were then prepared using standard protocols described above.

With phosphoramidite 17 in hand applicant synthesized several ribozymes with 2'-deoxy-2'-amino modifications. Analysis of the synthesis demonstrated coupling efficiency in 97-98% range. RNA cleavage activity of ribozymes containing 2'-deoxy-2'-amino-U modifications at U4 and/or U7 positions (see Figure 1), wherein the 2'-amino positions were eith r protected with Fmoc or Pht, was identical. Additionally, complete deprotection of 2'-deoxy-2'-amino-Uridine was confirmed by base-composition analysis. The coupling efficiency of phosphoramidite 17 was not effected over prolonged storage (1-2 months) at low temperatures.

Protecting 2' Position with a SEM Group

There follows a method using the 2'-(trimethylsilyl)ethoxymethyl protecting group (SEM) in the synthesis of oligoribonucleotides, and in particular those enzymatic molecules described above. For the synthesis of RNA it is important that the 2'-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the same time, this group should also be readily removed when desired. To that end the *t*-butyldimethylsilyl group has been efficacious (Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* **1990**, *18*, 5433-5441). However, long exposure times to tetra-*n*-butylammonium fluoride (TBAF) are generally required to fully remove this protecting group from the 2'-hydroxyl. In addition, the bulky alkyl substituents can prove to be a hindrance to coupling thereby necessitating longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic ammonia (Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* **1990**,

18, 5433-5441 and Stawinski,J.; Stromberg,R.; Thelin,M.; Westman,E. *Nucleic Acids Res.* **1988**, *16*, 9285-9298).

The (trimethylsilyl)ethoxymethyl ether (SEM) seems a suitable substitute. This protecting group is stable to base and all but the harsh st acidic conditions. Therefore it is stable under the conditions required for oligonucleotide synthesis. It can be readily introduced and the oxygen carbon bond makes it unable to migrate. Finally, the SEM group can be removed with $BF_3 \bullet OEt_2$ very quickly.

There follows a method for synthesis of RNA by protecting the 2'-position of a nucleotide during RNA synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group. The method can involve use of standard RNA synthesis conditions as discussed below, or any other equivalent steps. Those in the art are familiar with such steps. The nucleotide used can be any normal nucleotide or may be substituted in various positions by methods well known in the art, e.g., as described by Eckstein *et al.*, *International Publication* No. WO 92/07065, Perrault *et al.*, Nature **1990**, *344*, 565-568, Pieken *et al.*, Science **1991**, *253*, 314-317, Usman,N.; Cedergren,R.J. *Trends in Biochem. Sci.* **1992**, *17*, 334-339, Usman et al., PCT WO93/15187, and Sproat,B. *European Patent Application 92110298.4*.

This invention also features a method for covalently linking a SEM group to the 2'-position of a nucleotide. The method involves contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions. In a preferred embodiment, the conditions are dibutyltin oxide, tetrabutylammonium fluoride and SEM-Cl. Those in the art, however, will recognize that other equivalent conditions can also be used.

In another aspect, the invention features a method for removal of an SEM group from a nucleoside molecule or an oligonucleotide. The method involves contacting the molecule or oligonucleotide with boron trifluoride etherate ($BF_3 \bullet OEt_2$) under SEM removing conditions, *e.g.*, in acetonitrile.

Referring to Figure 18, there is shown the method for solid phase synthesis of RNA. A 2',5'-protected nucleotide is contacted with a solid phase bound nucleotide under RNA synthesis conditions to form a dinucleotide. The protecting group (R) at the 2'-position in prior art

methods can be a silyl ether, as shown in the Figure. In the method of the present invention, an SEM group is used in place of the silyl ether. Otherwise RNA synthesis can be performed by standard methodology.

Referring to Figure 19, there is shown the synthesis of 2'-*O*-SEM protected nucleosides and phosphoramadites. Briefly, a 5'-protected nucleoside (1) is protected at the 2'- or 3'-position by contacting with a derivative of SEM under appropriate conditions. Specifically, those conditions include contacting the nucleoside with dibutyltin oxide and SEM chloride. The 2 regioisomers are separated by chromatography and the 2'-protected moiety is converted into a phosphoramidite by standard procedure. The 3'-protected nucleoside is converted into a succinate derivative suitable for derivatization of a solid support.

Referring to Figure 20, a prior art method for deprotection of RNA using silyl ethers is shown. This contrasts with the method shown in Figure 21 in which deprotection of RNA containing an SEM group is performed. In step 1, the base protecting groups and cyanoethyl groups are removed by standard procedure. The SEM group is then removed as shown in the Figure. The details of the synthesis of phosphoramidites and SEM protected nucleosides and their use in synthesis of oligonucleotides and subsequent deprotection of

Example 14: Synthesis of 2'-*O*-((trimethylsilyl)ethoxymethyl)-5'-*O*- Dimethoxytrityl Uridine (2)

Referring to Figure 19, 5'-*O*-dimethoxytrityl uridine **1** (1.0 g, 1.83 mmol) in CH_3CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol) and TBAF (1 M, 2.38 mL, 2.38 mmol). The mixture was stirred for 2 h at RT (about 20-25°C) at which time (trimethylsilyl)ethoxymethyl chloride (SEM-Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred overnight and then filtered and evaporated. Flash chromatography (30% hexanes in ethyl acetate) yielded 347 mg (28.0%) of 2'-hydroxyl protected nucleoside **2** and 314 mg (25.3%) of 3'-hydroxyl protected nucleoside **3**.

Example 15: Synthesis of 2'-*O*-((trimethylsilyl)ethoxymethyl) Uridine (4)

Nucleoside **2** was detritylated following standard methods, as shown in Figure 19.

Example 16: Synthesis of 2'-*O*-((trimethylsilyl)ethoxymethyl)-5',3'-*O*-Acetyl Uridine (5)

Nucleoside **4** was acetylated following standard methods, as shown in Figure 19.

Example 17: Synthesis of 5',3'-*O*-Acetyl Uridine (6)

Referring to Figure 19, the fully protected uridine **5** (32 mg, 0.07 mmol) was dissolved in CH_3CN (700 µL) and $BF_3•OEt_2$ (17.5 µL, 0.14 mmol) was added. The reaction was stirred 15 m and MeOH was added to quench the reaction. Flash chromatography (5% MeOH in CH_2Cl_2) gave 20 mg (88%) of SEM deprotected nucleoside **6**.

Example 18: Synthesis of 2'-*O*-((trimethylsilyl)ethoxymethyl)-3'-*O*-Succinyl-5'-*O*- Dimethoxytrityl Uridine (2)

Nucleoside **3** was succinylated and coupled to the support following standard procedures, as shown in Figure 19.

Example 19: Synthesis of 2'-*O*-((trimethylsilyl)ethoxymethyl)-5'-*O*- Dimethoxytrityl Uridine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (8)

Nucleoside **3** was phosphitylated following standard methods, as shown in Figure 19.

Example 20: Synthesis of RNA Using 2'-*O*-SEM Protection

Referring to Figure 18, the method of synthesis used follows the general procedure for RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* **1990**, *18*, 5433-5441. The phosphoramidite **8** was coupled following standard RNA methods to provide a 10-mer of uridylic acid. Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 µmol scale protocol with a 10 m coupling step. A thirteen-fold excess (325 µL of 0.1 M = 32.5 µmol) of phosphoramidite and a 80-fold excess of tetrazole (400 µL of 0.5 M = 200 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, were 98-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-

Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I_2, 49 mM pyridine, 9% water in THF. Fisher Synth sis Grade acetonitrile was used directly from the reagent bottle.

Referring to Figure 21, the homopolymer was base deprotected with NH_3/EtOH at 65 °C. The solution was decanted and the support was washed twice with a solution of 1:1:1 H_2O:CH_3CN:MeOH. The combined solutions were dried down and then diluted with CH_3CN (1 mL). $BF_3 \cdot OEt_2$ (2.5 µL, 30 µmol) was added to the solution and aliquots were removed at ten time points. The results indicate that after 30 min deprotection is complete, as shown in Figure 22.

III. Vectors Expressing Ribozymes

There follows a method for expression of a ribozyme in a bacterial or eucaryotic cell, and for production of large amounts of such a ribozyme. In general, the invention features a method for preparing multi-copy cassettes encoding a defined ribozyme structure for production of a ribozyme at a decreased cost. A vector is produced which encodes a plurality of ribozymes which are cleaved at their 3' and 5' ends from an RNA transcript producted from the vector by only one other ribozyme. The system is useful for scaling up production of a ribozyme, which may be either modified or unmodified, *in situ* or *in vitro*. Such vector systems can be used to express a desired ribozyme in a specific cell, or can be used in an *in vitro* system to allow productiuon of large amounts of a desired riboqyne, The vectors of this invention allow a higher yield synthesis of a ribozyme in the form of an RNA transcript which is cleaved *in situ* or *in vitro* before or after transcript isolation.

Thus, this invention is distinct from the prior art in that a single ribozyme is used to process the 3' and 5' ends of each therapeutic, trans-acting or desired ribozyme instead of processing only one end, or only one ribozyme. This allows smaller vectors to be derived with multiple trans-acting ribozymes released by only one other ribozyme from the mRNA transcript. Applicant has also provided methods by which the activity of such ribozymes is increased compared to those in the art, by designing ribozyme-encoding vectors and the corresponding transcript such that

folding of the mRNA does not interfere with processing by the releasing ribozyme.

The stability of the ribozyme produced in this method can be enhanced by provision of sequences at the termini of the ribozymes as described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein.

The method of this invention is advantageous since it provides high yield synthesis of ribozymes by use of low cost transcription-based protocols, compared to existing chemical ribozyme synthesis, and can use isolation techniques currently used to purify chemically synthesized oligonucleotides. Thus, the method allows synthesis of ribozymes in high yield at low cost for analytical, diagnostic, or therapeutic applications.

The method is also useful for synthesis of ribozymes *in vitro* for ribozyme structural studies, enzymatic studies, target RNA accessibility studies, transcription inhibition studies and nuclease protection studies, much is described by Draper et al., PCT WO 93/23509 hereby incorporated by reference herein.

The method can also be used to produce ribozymes *in situ* either to increase the intracellular concentration of a desired therapeutic ribozyme, or to produce a concatameric transcript for subsequent *in vitro* isolation of unit length ribozyme. The desired ribozyme can be used to inhibit gene expression in molecular genetic analyses or in infectious cell systems, and to test the efficacy of a therapeutic molecule or treat afflicted cells.

Thus, in general, the invention features a vector which includes a bacterial, viral or eucaryotic promoter within a plasmid, cosmid, phagmid, virus, viroid, virusoid or phage vector. Other vectors are equally suitable and include double-stranded, or partially double-stranded DNA, formed by an amplification method such as the polymerase chain reaction, or double-stranded, partially double-stranded or single-stranded RNA, formed by site-directed homologous recombination into viral or viroid RNA genomes. Such vectors need not be circular. Transcriptionally linked to the promoter region is a first ribozyme-encoding region, and nucleotide sequences encoding a ribozyme cleavage sequence which is placed on either side of a region encoding a therapeutic or otherwise desired second ribozyme.

Suitable restriction endonuclease sites can be provided to ease construction of this vector in DNA vectors or in requisite DNA vectors of an RNA expression system. The desired second ribozyme may be any desired type of ribozyme, such as a hammerhead, hairpin , hepatitis delta virus (HDV) or other catalytic center, and can include group I and group II introns, as discussed above. The first ribozyme is chosen to cleave the encoded cleavage sequence, and may also be any desired ribozyme, for example, a *Tetrahymena* derived ribozyme, which may, for example, include an imbedded restriction endonuclease site in the center of a self-recognition sequence to aid in vector construction. This endonuclease site is useful for construction of the vector, and subsequent analysis of the vector.

When the promoter of such a vector is activated an RNA transcript is produced which includes the first and second ribozyme sequences. The first ribozyme sequence is able to act, under appropriate conditions, to cause cleavage at the cleavage sites to release the second ribozyme sequences. These second ribozyme sequences can then act at their target RNA sites, or can be isolated for later use or analysis.

Thus, in one aspect the invention features a vector which includes a first nucleic acid sequence (encoding a first ribozyme having intramolecular cleaving activity), and a second nucleic acid sequence (encoding a second ribozyme having intermolecular cleaving enzymatic activity) flanked by nucleic acid sequences encoding RNA which is cleaved by the first ribozyme to release the second ribozyme from the RNA transcript encoded by the vector. The second ribozyme may be flanked by the first ribozyme either on the 5' side or 3' side. If desired, the first ribozyme may be encoded on a separate vector and may have intermolecular cleaving activity.

As discussed above, the first ribozyme can be chosen to be any self-cleaving ribozyme, and the second ribozyme may be chosen to be any desired ribozyme. The flanking sequences are chosen to include sequences recognized by the first ribozyme. When the vector is caused to express RNA from these nucleic acid sequences, that RNA has the ability under appropriate conditions to cleave each of the flanking regions and thereby release one or more copies of th second ribozyme. If desired, several different second ribozymes can be produced by the same vector, or

several diff rent vectors can be placed in the same vessel or cell to produce different ribozymes.

In preferred embodiments, the vector includes a plurality of the nucleic acid sequences encoding the second ribozyme, each flanked by nucleic acid sequences recognized by the first ribozyme. Most preferably, such a plurality includes at least six to nine or even between 60 - 100 nucleic acid sequences. In other preferred embodiments, the vector includes a promoter which regulates expression of the nucleic acid encoding the ribozymes from the vector; and the vector is chosen from a plasmid, cosmid, phagmid, virus, viroid or phage. In a most preferred embodiment, the plurality of nucleic acid sequences are identical and are arranged in sequential order such that each has an identical end nearest to the promoter. If desired, a poly(A) sequence adjacent to the sequence encoding the first or second ribozyme may be provided to increase stability of the RNA produced by the vector; and a restriction endonuclease site adjacent to the nucleic acid encoding the first ribozyme is provided to allow insertion of nucleic acid encoding the second ribozyme during construction of the vector.

In a second aspect, the invention features a method for formation of a ribozyme expression vector by providing a vector including nucleic acid encoding a first ribozyme, as discussed above, and providing a single-stranded DNA encoding a second ribozyme, as discussed above. The single-stranded DNA is then allowed to anneal to form a partial duplex DNA which can be filled in by a treatment with an appropriate enzyme, such as a DNA polymerase in the presence of dNTPs, to form a duplex DNA which can then be ligated to the vector. Large vectors resulting from this method can then be selected to insure that a high copy number of the single-stranded DNA encoding the second ribozyme is incorporated into the vector.

In a further aspect, the invention features a method for production of ribozymes by providing a vector as described above, expressing RNA from that vector, and allowing cleavage by the first ribozyme to release the second ribozyme.

In preferred mbodiments, three different ribozym motifs are used as cis-cleaving ribozymes. The hammerhead, hairpin, and hepatitis delta

virus (HDV) ribozyme motifs consist of small, well-defined s quences that rapidly self-cleave *in vitro* (Symons, 1992 Annu. Rev. Biochem. 61, 641). While structural and functional differences exist among the three ribozyme motifs, they self-process efficiently *in vivo*. All three ribozyme motifs s lf-process to 87-95% completion in the absence of 3' flanking sequences. *In vitro*, the self-processing constructs described in this invention are significantly more active than those reported by Taira et al., 1990 supra; and Altschuler et al., 1992 Gene 122, 85. The present invention enables the use of cis-cleaving ribozymes to efficiently truncate RNA molecules at specific sites *in vivo* by ensuring lack of secondary structure which prevents processing.

Isolation of Therapeutic Ribozyme

The preferred method of isolating therapeutic ribozyme is by a chromatographic technique. The HPLC purification methods and reverse HPLC purification methods described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein, can be used. Alternatively, the attachment of complementary oligonucleotides to cellulose or other chromatography columns allows isolation of the therapeutic second ribozyme, for example, by hybridization to the region between the flanking arms and the enzymatic RNA. This hybridization will select against the short flanking sequences without the desired enzymatic RNA, and against the releasing first ribozyme. The hybridization can be accomplished in the presence of a chaotropic agent to prevent nuclease degradation. The oligonucleotides on the matrix can be modified to minimize nuclease activity, for example, by provision of 2'-O-methyl RNA oligonucleotides. Such modifications of the oligonucleotide attached to the column matrix will allow the multiple use of the column with minimal oligo degradation. Many such modifications are known in the art, but a chemically stable non-reducible modification is preferred. For example, phosphorothioate modifications can also be used.

The expressed ribozyme RNA can be isolated from bacterial or eucaryotic cells by routine procedures such as lysis followed by guanidine isothiocyanate isolation.

The current known self-cleaving site of *Tetrahymena* can be used in an alternative vector of this invention. If desired, the full-length

Tetrahymena sequence may be used, or a shorter sequence may be used. It is preferred that, in order to decrease the superfluous sequences in the self-cleaving site at the 5' cleavage end, the hairpin normally present in the *Tetrahymena* ribozyme should contain the therapeutic second ribozyme 3' sequence and its complement. That is, the first releasing ribozyme-encoding DNA is provided in two portions, separated by DNA encoding the desired second ribozyme. For example, if the therapeutic second ribozyme recognition sequence is CGGACGA/CGAGGA, then CGAGGA is provided in the self-cleaving site loop such that it is in a stem structure recognized by the *Tetrahymena* ribozyme. The loop of the stem may include a restriction endonuclease site into which the desired second ribozyme-encoding DNA is placed.

If desired, the vector may be used in a therapeutic protocol by use of the systems described by Lechner, PCT WO 92/13070, hereby incorporated by reference herein, to allow a timed expression of the therapeutic second ribozyme, as well as an appropriate shut off of cell or gene function. Thus, the vector will include a promoter which appropriately expresses enzymatically active RNA only in the presence of an RNA or another molecule which indicates the presence of an undesired organism or state. Such enzymatically active RNA will then kill or harm the cell in which it exists, as described by Lechner, id., or act to cause reduced expression of a desired protein product.

A number of suitable RNA vectors may also be used in this invention. The vectors include plant viroids, plant viruses which contain single or double-stranded RNA genomes and animal viruses which contain RNA genomes, such as the picornaviruses, myxoviruses, paramyxoviruses, hepatitis A virus, reovirus and retroviruses. In many instances cited, use of these viral vectors also results in tissue specific delivery of the ribozymes.

Example 21: Design of self-processing cassettes

In a preferred embodiment, applicant compared the *in vitro* and *in vivo* cis-cleaving activity of three different ribozyme motifs–the hammerhead, the hairpin and the hepatitis delta virus ribozyme–in order to assess their potential to process the ends of transcripts *in vivo*. To make a direct comparison among the three, however, it is important to design the ribozyme-containing transcripts to be as similar as possible. To this end,

all the ribozyme cassettes contained the sam trans-acting hammerhead ribozyme followed immediately by one of the three cis-acting ribozymes (Figure 23-25). For simplicity, applicant refers to each cassette by an abbreviation that indicates the downstream cis-cleaving ribozyme only. Thus HH refers to the cis-cleaving cassette containing a hammerhead ribozyme, while HP and HDV refer to the cassettes containing hairpin and hepatitis delta virus cis-cleaving ribozymes, respectively. The general design of the ribozyme cassettes, as well as specific differences among the cassettes, are outlined below.

A sequence predicted to form a stable stem-loop structure is included at the 5' end of all the transcripts. The hairpin stem contains the T7 RNA polymerase initiation sequence (Milligan & Uhlenbeck, 1989 Methods Enzymol. 180, 51) and its complement, separated be a stable tetra-loop (Antao et al., 1991 Nucleic Acids Res. 19, 5901). By incorporating the T7 initiation sequence into a stem-loop structure, applicant hoped to avoid nonproductive base pairing interactions with either the trans-acting ribozyme or with the cis-acting ribozyme. The presence of a hairpin at the end of a transcript may also contribute to the stability of the transcript *in vivo*. These are non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using a variety of promoters, initiator sequences and stem-loop structure combinations generally known in the art.

The trans-acting ribozyme used in this study is targeted to a site B (5'···CUGGAGUC↓GACCUUC···3'). The 5' binding arm of the ribozyme, 5'-GAAGGUC-3', and the core of the ribozyme, 5'-CUGAUGAGGCCGAAAGGCCGAA-3', remain constant in all cases. In addition, all transcripts also contain a single nucleotide between the 5' stem-loop and the first nucleotide of the ribozyme. The linker nucleotide was required to obtain the same activity *in vitro* that was measured with an identical ribozyme lacking the 5' hairpin. Because the three cis-cleaving ribozymes have different requirements at the site of cleavage, slight differences were unavoidable at the 3' end of the processed transcript. The junction between the trans- and cis-acting ribozyme is, however, designed so that there is minimal extraneous sequence left at the 3' end of the trans-cleaving ribozyme once cis-cleavage occurs. The only differences between the constructs lie in the 3' binding arm of the ribozyme, where

either 6 or 7 nucleotides, 5'-ACUCCA(+/-G)-3', complementary to the target sequence are present and where, after processing, two to five extra nucleotides remain.

The cis-cleaving hammerhead ribozyme used in the HH cassette is based on the design of Grosshans and Cech, 1991 supra. As shown in Figure 23, the 3' binding arm of the trans-acting ribozyme is included in the required base-pairing interactions of the cis-cleaving ribozyme to form stem I. Two extra nucleotides, UC, were included at the end of the 3' binding arm to form the self-processing hammerhead ribozyme site (Ruffner et al., 1990 supra) which remain on the 3' end of the trans-acting ribozyme following self-processing.

The hairpin ribozyme portion of the HP self-processing construct is based on the minimal wild-type sequence (Hampel & Tritz, 1989 supra). A tetra-loop at the end of helix 1 (3' side of the cleavage site) serves to link the two portions and thus allows a minimal five nucleotides to remain at the end of the released trans-acting ribozyme following self-processing. Two variants of HP were designed: HP(GU) and HP(GC). The HP(GU) was constructed with a G·U wobble base pair in helix 2 ($A_{52}G$ substitution; Figure 24). This slight destabilization of helix 2 was intended to improve self-processing activity by promoting product release and preventing the reverse reaction (Berzal-Herranz et al., 1992 Genes & Dev. 6, 129; Chowrira et al., 1993 Biochemistry 32, 1088). The HP(GC) cassette was constructed as a control for strong base-pairing interactions in helix 2 ($U_{77}C$ and $A_{52}G$ substitution; Figure 24). Another modification to discourage the reverse ligation reaction of the hairpin ribozyme was to shorten helix 1 (Figure 24) by one base pair relative to the wild-type sequence (Chowrira & Burke, 1991 Biochemistry 30, 8518).

The HDV ribozyme self-processes efficiently when the nucleotide 5' to the cleavage site is a pyrimidine, and somewhat less so when adenosine is in that position. No other sequence requirements have been identified upstream of the cleavage site, however, we have observed some decrease in activity when a stem-loop structure was present within 2 nt of the cleavage site. The HDV self-processing construct (Fig 25) was designed to generate the trans-acting hammerhead ribozyme with only two additional nucleotides at its 3' end after self-processing. The HDV sequence used here is based on the anti-genomic sequence (Perrota & Been, 1992 supra)

but includes the modifications of Been et al., 1992 (Biochemistry 31, 11843) in which cis-cleavage activity of the ribozyme was improved by the substitution of a shortened helix 4 for a wild-type stem-loop (Figure 25).

To prepare DNA inserts that encode self-processing ribozyme cassettes, partially overlapping top- and bottom-strand oligonucleotides (60-90 nucleotides) were designed to include sequences for the T7 promoter, the trans-acting ribozyme, the cis-cleaving ribozyme and appropriate restriction sites for use in cloning (see Fig. 26). The single-strand portions of annealed oligonucleotides were converted to double-strands using Sequenase® (U.S. Biochemicals). Insert DNA was ligated into *EcoR1/Hin*dIII-digested puc18 and transformed into *E. coli* strain DH5α using standard protocols (Maniatis et al., 1982 in Molecular Cloning Cold Spring Harbor Press). The identity of positive clones was confirmed by sequencing small-scale plasmid preparations.

Larger scale preparations of plasmid DNA for use as *in vitro* transcription templates and in transactions were prepared using the protocol and columns from QIAGEN Inc. (Studio City, CA) except that an additional ethanol precipitation was included as the final step.

Example 22: RNA Processing *in vitro*

Transcription reactions containing linear plasmid templates were carried out essentially as described (Milligan & Uhlenbeck, 1989 Supra; Chowrira & Burke, 1991 Supra). In order to prepare 5' end-labeled transcripts, standard transcription reactions were carried out in the presence of 10-20 µCi [γ-^{32}P]GTP, 200 µM each NTP and 0.5 to 1 µg of linearized plasmid template. The concentration of $MgCl_2$ was maintained at 10 mM above the total nucleotide concentration.

To compare the ability of the different ribozyme cassettes to self-process *in vitro*, each construct was transcribed and allowed to undergo self-processing under identical conditions at 37°C. For these comparisons, equal amounts of linearized DNA templates bearing the various ribozyme cassettes were transcribed in the presence of [γ-^{32}P]GTP to generate 5' end-labeled transcripts. In this manner only the full-length, unprocessed transcripts and the released trans-ribozymes are visualized by autoradiography. In all reactions, Mg^{2+} was included at 10 mM above the nucleotide concentration so that cleavage by all the ribozyme cassettes

would be supported. Transcription templates were linearized at several positions by digestion with different restriction enzymes so that self-processing in the presence of increasing lengths of downstream sequence could be compared (see Fig. 26). The resulting transcripts have either 4-5 non-ribozyme nucleotides at the 3' end (*Hin*dIII-digested template), 220 nucleotides (*Nde*I digested templates) or 454 nucleotides of downstream sequence (*Rca*I digested template).

As shown in Figure 27, all four ribozyme cassettes are capable of self-processing and yield RNA products of expected sizes. Two nucleotides essential for hammerhead ribozyme activity (Ruffner et al., 1990 supra) have been changed in the HH(mutant) core sequence (see Figure 23) and so this transcript is unable to undergo self-processing (Fig. 27). This is evidenced by the lack of a released 5' RNA in the HH(mutant), although the full-length RNAs are present . Comparison of the amounts of released trans-ribozyme (Fig. 27) indicate that there are differences in the ability of these ribozymes to self-process *in vitro*, especially with respect to the presence of downstream sequence. For the two HP constructs, it is clear that HP(GC) is more efficient than the HP(GU) ribozyme, both in the presence and in the absence of extra downstream sequence. In addition, the activity of HP(GU) falls off more dramatically when downstream sequence is present. The stronger G:C base pair likely contributes to the HP(GC) construct's ability to fold correctly (and/or more quickly) into the productive structure, even when as much as 216 extra nucleotides are present downstream. The HH ribozyme construct is also quite efficient at self-processing, and slightly better than the HP(GU) construct even when downstream sequence is present.

Of the three ribozyme motifs, the presence of extra downstream sequence seems to most affect the efficiency of HDV. When no extra sequence is present downstream, HDV is quite efficient and self-processes to approximately the same level as the HH and HP(GC) cassettes. However, when extra downstream sequence is present, the self-processing activity seems to decrease almost as dramatically as is seen with the (sub-optimal) HP(GU) cassette.

Example 23: Kinetics of self-processing reaction

*Hin*dIII-digested template (250 ng) was used in a standard transcription reaction mixture containing: 50 mM Tris·HCl pH 8.3; 1 mM ATP, GTP and UTP; 50 µM CTP; 40 µCi [α-^{32}P]CTP; 12 mM $MgCl_2$; 10 mM DTT. The transcription/self-processing reaction was initiated by the addition of T7 RNA polymerase (15 U/µl). Aliquots of 5 µl were taken at regular time intervals and the reaction was stopped by adding an equal volume of 2x formamide loading buffer (95% formamide, 15 mM EDTA, & dyes) and freezing on dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel and results were quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Ribozyme self-cleavage rates were determined from non-linear, least-squares fits (KaleidaGraph, Synergy Software,Reeding, PA) of the data to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt}(1-e^{-kt})$$

where t represents time and k represents the unimolecular rate constant for cleavage (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977).

Linear templates were prepared by digesting the plasmids with *Hin*dIII so that transcripts will contain only four to five vector-derived nucleotides at the 3' end (see Figure 23-25). By comparison of the unimolecular rate constant (k) determined for each construct, it is clear that HH is the most efficient at self-processing (Table 44). The HH transcript self-processes 2-fold faster than HDV and 3-fold faster than HP(GC) transcripts. Although the HP(GU) RNA undergoes self-processing, it is at least 6-fold slower than the HP(GC) construct. This is consistent with previous observations that the stability of helix 2 is essential for self-processing and trans-cleavage activity of the hairpin ribozyme (Hampel et al., 1990 supra; Chowrira & Burke, 1991 supra). The rate of HH self-cleavage during transcription measured here (1.2 min^{-1}) is similar to the rate measured by Long and Uhlenbeck 1994 supra using a HH that has a different stem I and stem III. Self-processing rates during transcription for HP and HDV have not been previously reported. However, self-processing of the HDV ribozyme–as measured here during transcription–is significantly slower than when tested after isolation from a denaturing gel (Been et al., 1992 supra). This decrease likely reflects the difference in protocol as well as the presence of 5' flanking sequence in the HDV construct used here.

Example 24: Effect of downstream sequences on trans-cleavage *in vitro*

Transcripts containing the trans ribozyme with or without 3' flanking sequences were assayed for their ability to cleave their target in trans. To this end, transcripts from three templates were resolved on a preparative gel and bands corresponding both to processed trans-acting ribozymes from the HH transcription reaction, and to full-length HH(mutant) and ΔHDV transcripts were isolated. In all three transcripts the trans-acting ribozyme portion is identical--with the exception of sequences at their 3' ends. The HH trans-acting ribozyme contains only an additional UC at its 3' end, while HH(mutant) and ΔHDV have 52 and 37 nucleotides, respectively, at their 3' ends. A 622 nucleotide, internally-labeled target RNA was incubated, under ribozyme excess conditions, along with the three ribozyme transcripts in a standard reaction buffer.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 622 nt region (containing hammerhead site P) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α-^{32}P]CTP (Chowrira & Burke, 1991 supra). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 µl DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1µM) and internally labeled 622 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris·HCl pH 7.5 and 10 mM $MgCl_2$) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 µl were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager® (Molecular Dynamics, Sunnyvale, CA).

The HH trans-acting ribozyme cleaves the target RNA approximately 10-fold faster than the ΔHDV transcript and greater than 20-fold faster than

the HH(mutant) transcript (Figure 28). The additional nucleotides at the end of HH(mutant) form 7 base-pairs with the 3' target-binding arm of the trans-acting ribozyme (Figure 23). This interaction must be disrupted (at a cost of 6 kcal/mole) to make the trans-acting ribozyme available for binding the target sequence. In contrast, the additional nucleotides at the end of ΔHDV were not designed to form any strong, alternative base-pairing with the trans-ribozyme. Nevertheless, the ΔHDV sequences are predicted to form multiple structures involving the 3' target-binding arm of the trans ribozyme that have stabilities ranging from 1-2 kcal/mole. Thus, the observed reductions in activity for the ΔHDV and HH(mutant) constructs are consistent with the predicted folded structures, and it reinforces the view that the flanking sequences can decrease the catalytic efficiency of a ribozyme through nonproductive interactions with either the ribozyme or the substrate or both.

Example 25: RNA self-processing *in vivo*

Since three of the constructs (HH, HDV and HP(GC)) self-process efficiently in solution, the affect of the mammalian cellular milieu on ribozyme self-processing was next explored by applicant. A transient expression system was employed to investigate ribozyme activity *in vivo*. A mouse cell line (OST7-1) that constitutively expresses T7 RNA polymerase in the cytoplasm was chosen for this study (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA 87, 6743). In these cells plasmids containing a ribozyme cassette downstream of the T7 promoter will be transcribed efficiently in the cytoplasm (Elroy-Stein & Moss, 1990 supra).

Monolayers of a mouse L9 fibroblast cell line (OST7-1; Elroy-Stein and Moss, 1990 supra) were grown in 6-well plates with ~ 5×10^5 cells/well. Cells were transfected with circular plasmids (5 µg/well) using the calcium phosphate-DNA precipitation method (Maniatis et al., 1982 supra). Cells were lysed (4 hours post-transfection) by the addition of standard lysis buffer (200 µl/well) containing 4M guanadinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl (Chomczynski and Sacchi, 1987 Anal. Biochem. 162, 156), and 50 mM EDTA pH 8.0. The lysate was extracted once with water-saturated phenol followed by one extraction with chloroform:isoamyl alcohol (25:1). Total cellular RNA was precipitated with an equal volume of isopropanol. The RNA pellet was resuspended in 0.2

M ammonium acetate and reprecipitated with ethanol. The pellet was then washed with 70% ethanol and resuspended in DEPC-treated water.

Purified cellular RNA (3 µg/reaction) was first denatured in the presence of a 5' end-labeled DNA primer (100 pmol) by heating to 90°C for 2 min. in the absence of Mg^{2+}, and then snap-cooling on ice for at least 15 min. This protocol allows for efficient annealing of the primer to its complementary RNA sequence. The primer was extended using Superscript II reverse transcriptase (8 U/µl; BRL) in a buffer containing 50 mM Tris·HCl pH 8.3; 10 mM DTT; 75 mM KCl; 1 mM $MgCl_2$; 1 mM each dNTP. The extension reaction was carried out at 42°C for 10 min. The reaction was terminated by adding an equal volume of 2x formamide gel loading buffer and freezing on crushed dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel. The primer sequences are as follows: HH primer, 5'-CTCCAGTTTCGAGCTTT-3'; HDV primer, 5'-AAGTAGCCCAGGTCGGACC-3'; HP primer, 5'-ACCAGGTAATATACCACAAC-3'.

As shown in Figure 29, specific bands corresponding to full-length precursor RNA and 3' cleavage products were detected from cells transfected with the self-processing cassettes. All three constructs, in addition to being transcriptionally active, appear to self-process efficiently in the cytoplasm of OST7-1 cells. In particular, the HH and HP(GC) constructs self-process to greater than 95%. The overall extent of self-processing in OST7-1 cells appears to be strikingly similar to the extent of self-processing *in vitro* (Figure 29 "In Vitro +$MgCl_2$" vs. "Cellular").

Consistent with the *in vitro* self-processing results, the HP(GU) cassette self-processed to approximately 50% in OST7-1 cells. As expected, transfection with plasmids containing the HH(mutant) cassette yielded a primer-extension product corresponding to the full-length RNA with no detectable cleavage products (Figure 29). The latter result strongly suggests that the primer extension band corresponding to the 3' cleavage product is not an artifact of reverse transcription.

Applicant was concerned with the possibility that RNA self-processing might occur during cell lysis, RNA isolation and /or the primer extension assay. Two precautions were taken to exclude this possibility. First, 50 mM EDTA was included in the lysis buffer. EDTA is a strong chelator of divalent

metal ions such as Mg^{2+} and Ca^{2+} that are necessary for ribozyme activity. Divalent metal ions are therefore unavailable to self-processing RNAs following cell lysis. A second precaution involved using primers in the primer-extension assay that were designed to hybridize to essential regions of the processing ribozyme. Binding of these primers should prevent the 3' cis-acting ribozymes from folding into the conformation essential for catalytic activity.

Two experiments were carried out to further eliminate the possibility that self-processing is occurring either during RNA preparations or during the primer extension analysis. The first experiment involves primer extension analysis on full-length precursor RNAs that were added to non-transfected OST7-1 lysates after cell lysis. Thus, only if self-processing is occurring at some point after lysis would cleavage products be detected. Full-length precursor RNAs were prepared by transcribing under conditions of low Mg^{2+} (5 mM) and high NTP concentration (total 12 mM) in an attempt to eliminate the free Mg^{2+} required for the self-processing reaction (Michel et al. 1992 Genes & Dev. 6, 1373). The full-length precursor RNAs were gel-purified, and a known amount was added to lysates of non-transfected OST7-1 cells. RNA was purified from these lysates and incubated for 1 hr in DEPC-treated water at 37° C prior to the standard primer extension analysis (Figure 29, *in vitro* "–$MgCl_2$" control). The predominant RNA detected in all cases corresponds to the primer extension product of full-length precursor RNAs. If, instead, the purified RNA containing the full-length precursor is incubated in 10 mM $MgCl_2$ prior to the primer extension analysis, most or all of the RNA detected by primer extension analysis undergoes cleavage (Figure 29, *in vitro* "+$MgCl_2$" control). These results indicate that the standard RNA isolation and primer extension protocols used here do not provide a favorable environment for RNA self-processing, even though the RNA in question is inherently able to undergo self-cleavage.

In a second experiment to demonstrate lack of self-processing during work up, internally-labeled precursor RNAs were prepared and added to non-transfected OST7-1 lysates as in the previous control. The internally-labeled precursor RNAs were carried through the RNA purification and primer extension reactions (in the presence of unlabeled primers) and analyzed to determine the extent of self-processing. By this analysis, the

vast majority of th added full-length RNA remained intact during the entire process of RNA isolation and primer extension.

These two control experiments validate the protocols used and support applicant's conclusion that the self-processing reactions catalyzed by HH, HDV and HP(GC) cassettes are occurring in the cytoplasm of OST7-1 cells.

Sequences in figures 23 through 25 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

In addition, those in the art will recognize that Applicant provides guidance through the above examples as to how to best design vectors of this invention so that secondary structure of the mRNA allows efficient cleavage by releasing ribozymes. Thus, the specific constructs are not limiting in this invention. Such constructs can be readily tested as described above for such secondary structure, either by computer folding algorithms or empirically. Such constructs will then allow at least 80% completion of release of ribozymes, which can be readily determined as described above or by methods known in the art. That is, any such secondary structure in the RNA does not reduce release of the ribozymes by more than 20%.

IV. Ribozymes Expressed by RNA Polymerase III

Applicant has determined that the level of production of a foreign RNA, using a RNA polymerase III (pol III) based system, can be significantly enhanced by ensuring that the RNA is produced with the 5' terminus and a 3' region of the RNA molecule base-paired together to form a stable intramolecular stem structure. This stem structure is formed by hydrogen bond interactions (either Watson-Crick or non-Watson-Crick) between nucleotides in the 3' region (at least 8 bases) and complementary nucleotides in the 5' terminus of the same RNA molecule.

Although the example provided below involves a type 2 pol III gene unit, a number of other pol III promoter systems can also be used, for example, tRNA (Hall et al., 1982 *Cell* 29, 3-5), 5S RNA (Nielsen et al., 1993, *Nucleic Acids Res.* 21, 3631-3636), ad novirus VA RNA (Fowlkes and Shenk, 1980 *Cell* 22, 405-413), U6 snRNA (Gupta and Reddy, 1990

Nucleic Acids Res. 19, 2073-2075), vault RNA (Kickoefer et al., 1993 *J. Biol. Chem.* 268, 7868-7873), telomerase RNA (Romero and Blackburn, 1991 *Cell* 67, 343-353), and others.

The construct described in this invention is able to accumulate RNA to a significantly higher level than other constructs, even those in which 5' and 3' ends are involved in hairpin loops. Using such a construct the level of expression of a foreign RNA can be increased to between 20,000 and 50,000 copies per cell. This makes such constructs, and the vectors encoding such constructs, excellent for use in decoy, therapeutic editing and antisense protocols as well as for ribozyme formation. In addition, the molecules can be used as agonist or antagonist RNAs (affinity RNAs). Generally, applicant believes that the intramolecular base-paired interaction between the 5' terminus and the 3' region of the RNA should be in a double-stranded structure in order to achieve enhanced RNA accumulation.

Thus, in one preferred embodiment the invention features a pol III promoter system (e.g., a type 2 system) used to synthesize a chimeric RNA molecule which includes tRNA sequences and a desired RNA (e.g., a tRNA-based molecule).

The following exemplifies this invention with a type 2 pol III promoter and a tRNA gene. Specifically to illustrate the broad invention, the RNA molecule in the following example has an A box and a B box of the type 2 pol III promoter system and has a 5' terminus or region able to base-pair with at least 8 bases of a complementary 3' end or region of the same RNA molecule. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using other pol III promoter systems and techniques generally known in the art.

By "terminus" is meant the terminal bases of an RNA molecule, ending in a 3' hydroxyl or 5' phosphate or 5' cap moiety. By "region" is meant a stretch of bases 5' or 3' from the terminus that are involved in base-paired interactions. It need not be adjacent to the end of the RNA. Applicant has determined that base pairing of at least one end of the RNA molecule with a region not more than about 50 bases, and preferably only 20 bases, from

the other end of the molecule provides a useful molecule able to be expressed at high levels.

By "3' region" is meant a stretch of bases 3' from the terminus that are involved in intramolecular bas-paired interaction with complementary nucleotides in the 5' terminus of the same molecule. The 3' region can be designed to include the 3' terminus. The 3' region therefore is ≥ 0 nucleotides from the 3' terminus. For example, in the S35 construct described in the present invention (Fig. 40) the 3' region is one nucleotide from the 3' terminus. In another example, the 3' region is ~ 43 nt from 3' terminus. These examples are not meant to be limiting. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. Generally, it is preferred to have the 3' region within 100 bases of the 3' terminus.

By "tRNA molecule" is meant a type 2 pol III driven RNA molecule that is generally derived from any recognized tRNA gene. Those in the art will recognize that DNA encoding such molecules is readily available and can be modified as desired to alter one or more bases within the DNA encoding the RNA molecule and/or the promoter system. Generally, but not always, such molecules include an A box and a B box that consist of sequences which are well known in the art (and examples of which can be found throughout the literature). These A and B boxes have a certain consensus sequence which is essential for a optimal pol III transcription.

By "chimeric tRNA molecule" is meant a RNA molecule that includes a pol III promoter (type 2) region. A chimeric tRNA molecule, for example, might contain an intramolecular base-paired structure between the 3' region and complementary 5' terminus of the molecule, and includes a foreign RNA sequence at any location within the molecule which does not affect the activity of the type 2 pol III promoter boxes. Thus, such a foreign RNA may be provided at the 3' end of the B box, or may be provided in between the A and the B box, with the B box moved to an appropriate location either within the foreign RNA or another location such that it is effective to provide pol III transcription. In one example, the RNA molecule may include a hammerhead ribozyme with the B box of a type 2 pol III promoter provided in stem II of the ribozyme. In a second example, the B box may be provided in stem IV region of a hairpin ribozyme. A specific example of such RNA molecules is provided below. Those in the art will

recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "desired RNA" molecule is meant any foreign RNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such molecules include antisense RNA molecules, decoy RNA molecules, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA.

By "antisense RNA" is meant a non-enzymatic RNA molecule that binds to another RNA (target RNA) by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi et al., 1991 Annu. Rev. Biochem. 60, 631-652). By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, 1988 J.*American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

By "decoy RNA" is meant an RNA molecule that mimics the natural binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV *tat* protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger et al., 1990 Cell 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "therapeutic editing RNA" is meant an antisense RNA that can bind to its cellular target (RNA or DNA) and mediate the modification of a specific base.

By "agonist RNA" is meant an RNA molecule that can bind to protein receptors with high affinity and cause the stimulation of specific cellular pathways.

By "antagonist RNA" is meant an RNA molecule that can bind to cellular proteins and prevent it from performing its normal biological function (for example, s_e Tsai et al., 1992 *Proc. Natl. Acad. Sci.* USA 89, 8864-8868).

In other aspects, the invention includes vectors encoding RNA molecules as described above, cells including such vectors, methods for producing the desired RNA, and use of the vectors and cells to produce this RNA.

Thus, the invention features a transcribed non-naturally occuring RNA molecule which includes a desired therapeutic RNA portion and an intramolecular stem formed by base-pairing interactions between a 3' region and complementary nucleotides at the 5' terminus in the RNA. The stem preferably includes at least 8 base pairs, but may have more, for example, 15 or 16 base pairs.

In preferred embodiments, the 5' terminus of the chimeric tRNA includes a portion of the precursor molecule of the primary tRNA molecule, of which ≥ 8 nucleotides are involved in base-pairing interaction with the 3' region; the chimeric tRNA contains A and B boxes; natural sequences 3' of the B box are deleted, which prevents endogenous RNA processing; the desired RNA molecule is at the 3' end of the B box; the desired RNA molecule is between the A and the B box; the desired RNA molecule includes the B box; the desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA; the molecule has an intramolecular stem resulting from a base-paired interaction between the 5' terminus of the RNA and a complementary 3' region within the same RNA, and includes at least 8 bases; and the 5' terminus is able to base pair with at least 15 bases of the 3' region.

In most preferred embodiments, the molecule is transcribed by a RNA polymerase III based promoter system, *e.g.*, a type 2 pol III promoter system; the molecule is a chimeric tRNA, and may have the A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases; DNA vector encoding the RNA molecule of claim 51.

In other related aspects, the invention features an RNA or DNA vector encoding the above RNA molecule, with the portions of the vector encoding the RNA functioning as a RNA pol III promoter; or a cell containing the vector ; or a method to provide a desired RNA molecule in a cell, by introducing the molecule into a cell with an RNA molecule as described above. The cells can be derived from animals, plants or human beings.

In order for RNA-based gene therapy approaches to be effective, sufficient amounts of the therapeutic RNA must accumulate in the appropriate intracellular compartment of the treated cells. Accumulation is a function of both promoter strength of the antiviral gene, and the intracellular stability of the antiviral RNA. Both RNA polymerase II (pol II) and RNA polymerase III (pol III) based expression systems have been used to produce therapeutic RNAs in cells (Sarver & Rossi, 1993 *AIDS Res. & Human Retroviruses* 9, 483-487; Yu et al., 1993 *P.N.A.S.*(USA) 90, 6340-6344). However, pol III based expression cassettes are theoretically more attractive for use in expressing antiviral RNAs for the following reasons. Pol II produces messenger RNAs located exclusively in the cytoplasm, whereas pol III produces functional RNAs found in both the nucleus and the cytoplasm. Pol II promoters tend to be more tissue restricted, whereas pol III genes encode tRNAs and other functional RNAs necessary for basic "housekeeping" functions in all cell types. Therefore, pol III promoters are likely to be expressed in all tissue types. Finally, pol III transcripts from a given gene accumulate to much greater levels in cells relative to pol II genes.

Intracellular accumulation of therapeutic RNAs is also dependent on the method of gene transfer used. For example, the retroviral vectors presently used to accomplish stable gene transfer, integrate randomly into the genome of target cells. This random integration leads to varied expression of the transferred gene in individual cells comprising the bulk treated cell population. Therefore, for maximum effectiveness, the transferred gene must have the capacity to express therapeutic amounts of the antiviral RNA in the entire treated cell population, regardless of the integration site.

Pol III System

The following is just one non-limiting example of the invention. A pol III based genetic element derived from a human tRNAi^{met} gene and termed Δ3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), has been adapted to express antiviral RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523). This element was inserted into the DC retroviral vector (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523) to accomplish stable gene transfer, and used to express antisense RNAs against moloney murine leukemia virus and anti-HIV decoy RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523; Sullenger et al., 1990 *Cell* 63, 601-608; Sullenger et al., 1991 *J. Virol.* 65, 6811-6816; Lee et al., 1992 *The New Biologist* 4, 66-74). Clonal lines are expanded from individual cells present in the bulk population, and therefore express similar amounts of the therapeutic RNA in all cells. Development of a vector system that generates therapeutic levels of therapeutic RNA in all treated cells would represent a significant advancement in RNA based gene therapy modalities.

Applicant examined hammerhead (HHI) ribozyme (RNA with enzymatic activity) expression in human T cell lines using the Δ3-5 vector system (These constructs are termed "Δ3-5/HHI"; Fig. 34). On average, ribozymes were found to accumulate to less than 100 copies per cell in the bulk T cell populations. In an attempt to improve expression levels of the Δ3–5 chimera, the applicant made a series of modified Δ3-5 gene units containing enhanced promoter elements to increase transcription rates, and inserted structural elements to improve the intracellular stability of the ribozyme transcripts (Fig. 34). One of these modified gene units, termed S35, gave rise to more than a 100-fold increase in ribozyme accumulation in bulk T cell populations relative to the original Δ3-5/HHI vector system. Ribozyme accumulation in individual clonal lines from the pooled T cell populations ranged from 10 to greater than 100 fold more than those achieved with the original Δ3-5/HHI version of this vector.

The S35 gene unit may be used to express other therapeutic RNAs including, but not limited to, ribozymes, antisense, decoy, therapeutic editing, agonist and antagonist RNAs. Application of the S35 gene unit would not be limited to antiviral therapies, but also to other diseases, such as cancer, in which therapeutic RNAs may be effective. The S35 gene unit may be used in the context of other vector systems besides retroviral

vectors, including but not limited to, other stable gene transfer systems such as adeno-associated virus (AAV; Carter, 1992 *Curr. Opin. Genet. Dev.* 3, 74), as well as transient vector systems such as plasmid delivery and adenoviral vectors (Berkner, 1988 *BioTechniques* 6, 616-629).

As described below, the S35 vector encodes a truncated version of a tRNA wherein the 3' region of the RNA is base-paired to complementary nucleotides at the 5' terminus, which includes the 5' precursor portion that is normally processed off during tRNA maturation. Without being bound by any theory, Applicant believes this feature is important in the level of expression observed. Thus, those in the art can now design equivalent RNA molecules with such high expression levels. Below are provided examples of the methodology by which such vectors and tRNA molecules can be made.

Δ3-5 Vectors

The use of a truncated human $tRNA_i^{met}$ gene, termed Δ3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), to drive expression of antisense RNAs, and subsequently decoy RNAs (Sullenger et al., 1990 *supra*) has recently been reported. Because tRNA genes utilize internal pol III promoters, the antisense and decoy RNA sequences were expressed as chimeras containing $tRNA_i^{met}$ sequences. The truncated tRNA genes were placed into the U3 region of the 3' moloney murine leukemia virus vector LTR (Sullenger et al., 1990 *supra*).

Base-Paired Structures

Since the Δ3-5 vector combination has been successfully used to express inhibitory levels of both antisense and decoy RNAs, applicant cloned ribozyme-encoding sequences (termed as "Δ3-5/HHI") into this vector to explore its utility for expressing therapeutic ribozymes. However, low ribozyme accumulation in human T cell lines stably transduced with this vector was observed (Fig. 35). To try and improve accumulation of the ribozyme, applicant incorporated various RNA structural elements (Fig. 34) into one of the ribozyme chimeras (Δ3-5/HHI).

Two strategies were used to try and protect the termini of the chimeric transcripts from exonucleolytic degredation. One strategy involved the incorporation of stem-loop structures into the termini of the transcript. Two

such constructs were cloned, S3 which contains a stem-loop structure at the 3' end, and S5 which contains stem-loop structures at both ends of the transcript (Figure 34). The second strategy involved modification of the 3' terminal sequences such that the 5' terminus and the 3' end sequences can form a stable base-paired stem. Two such constructs were made: S35 in which the 3' end was altered to hybridize to the 5' leader and acceptor stem of the $tRNA_i^{met}$ domain, and S35Plus which was identical to S35 but included more extensive structure formation within the non-ribozyme portion of the Δ3–5 chimeras (Figure 34). These stem-loop structures are also intended to sequester non-ribozyme sequences in structures that will prevent them from interfering with the catalytic activity of the ribozyme. These constructs were cloned, producer cell lines were generated, and stably-transduced human MT2 (Harada et al., 1985 *supra*) and CEM (Nara & Fischinger, 1988 *supra*) cell lines were established (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). The RNA sequences and structure of S35 and S35 Plus are provided in Figures 40-47.

Referring to Figure 48, there is provided a general structure for a chimeric RNA molecule of this invention. Each N independently represents none or a number of bases which may or may not be base paired. The A and B boxes are optional and can be any known A or B box, or a consensus sequence as exemplified in the figure. The desired nucleic acid to be expressed can be any location in the molecule, but preferably is on those places shown adjacent to or between the A and B boxes (designated by arrows). Figure 49 shows one example of such a structure in which a desired RNA is provided 3' of the intramolecular stem. A specific example of such a construct is provided in Figures 50 and 51.

Example 26: Cloning of Δ3-5-Ribozyme Chimera

Oligonucleotides encoding the S35 insert that overlap by at least 15 nucleotides were designed (5' GATCCACTCTGCTGTTCTGTTTTTGA 3' and 5' CGCGTCAAAAACAGAACAGCAGAGTG 3'). The oligonucleotides (10 µM each) were denatured by boiling for 5 min in a buffer containing 40 mM Tris.HCl, pH8.0. The oligonucleotides were allowed to anneal by snap cooling on ice for 10-15 min.

The annealed oligonucleotide mixture was converted into a double-stranded molecule using Sequenase® enzyme (US Biochemicals) in a

buffer containing 40 mM Tris.HCl, pH7.5, 20 mM $MgCl_2$, 50 mM NaCl, 0.5 mM each of th four deoxyribonucleotide triphosphates, 10 mM DTT. The reaction was allowed to proceed at 37°C for 30 min. The reaction was stopped by heating to 70°C for 15 min.

The double stranded DNA was digested with appropriate restriction endonucleases (*Bam*HI and *Mlu*I) to generate ends that were suitable for cloning into the Δ3-5 vector.

The double-stranded insert DNA was ligated to the Δ3-5 vector DNA by incubating at room temperature (about 20°C) for 60 min in a buffer containing 66 mM Tris.HCl, pH 7.6, 6.6 mM $MgCl_2$, 10 mM DTT, 0.066 μM ATP and 0.1U/μl T4 DNA Ligase (US Biochemicals).

Competent *E. coli* bacterial strain was transformed with the recombinant vector DNA by mixing the cells and DNA on ice for 60 min. The mixture was heat-shocked by heating to 37°C for 1 min. The reaction mixture was diluted with LB media and the cells were allowed to recover for 60 min at 37°C. The cells were plated on LB agar plates and incubated at 37°C for ~ 18 h.

Plasmid DNA was isolated from an overnight culture of recombinant clones using standard protocols (Ausubel et al., *Curr. Protocols Mol. Biology* 1990, Wiley & Sons, NY).

The identity of the clones were determined by sequencing the plasmid DNA using the Sequenase® DNA sequencing kit (US Biochemicals).

The resulting recombinant Δ3-5 vector contains the S35 sequence. The HHI encoding DNA was cloned into this Δ3-5-S35 containing vector using *Sac*II and *Bam*HI restriction sites.

Example 27: Northern analysis

RNA from the transduced MT2 cells were extracted and the presence of Δ3-5/ribozyme chimeric transcripts were assayed by Northern analysis (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Northern analysis of RNA extracted from MT2 transductants showed that Δ3-5/ribozyme chimeras of appropriate sizes were expressed (Fig. 35,36). In addition, these results demonstrated the relative differences in accumulation among the different constructs (Figure 35,36). The pattern of

expression seen from the Δ3-5/HHI ribozyme chimera was similar to 12 other ribozym s cloned into the Δ3-5 vector (not shown). In MT-2 cell line, Δ3-5/HHI ribozyme chimeras accumulated, on average, to less than 100 copies per cell.

Addition of a stem-loop onto the 3' end of Δ3-5/HHI did not lead to increased Δ3–5 levels (S3 in Fig. 35,36). The S5 construct containing both 5' and 3' stem-loop structures also did not lead to increased ribozyme levels (Fig. 35,36).

Interestingly, the S35 construct expression in MT2 cells was about 100-fold more abundant relative to the original Δ3-5/HHI vector transcripts (Fig. 35,36). This may be due to increased stability of the S35 transcript.

Example 28: Cleavage activity

To assay whether ribozymes transcribed in the transduced cells contained cleavage activity, total RNA extracted from the transduced MT2 T cells were incubated with a labeled substrate containing the HHI cleavage site (Figure 37). Ribozyme activity in all but the S35 constructs, was too low to detect. However, ribozyme activity was detectable in S35-transduced T cell RNA. Comparison of the activity observed in the S35-transduced MT2 RNA with that seen with MT2 RNA in which varying amounts of in vitro transcribed S5 ribozyme chimeras, indicated that between 1-3 nM of S35 ribozyme was present in S35-transduced MT2 RNA. This level of activity corresponds to an intracellular concentration of 5,000-15,000 ribozyme molecules per cell.

Example 29: Clonal variation

Variation in the ribozyme expression levels among cells making up the bulk population was determined by generating several clonal cell lines from the bulk S35 transduced CEM line (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY) and the ribozyme expression and activity levels in the individual clones were measured (Figure 38 and 39). All the individual clones were found to express active ribozyme. The ribozyme activity detected from each clone correlated well with the relative amounts of ribozyme observed by Northern analysis. Steady state ribozyme lev ls among the clon s ranged from approximately 1,000 molecules per cell in clone G to 11,000 molecules per cell in clone H (Fig.

38). The mean accumulation among the clones, calculated by averaging the ribozyme levels of the clones, exactly equaled the level measured in the parent bulk population. This suggests that the individual clones are representative of the variation present in the bulk population.

The fact that all 14 clones were found to express ribozyme indicate that the percentage of cells in the bulk population expressing ribozyme is also very high. In addition, the lowest level of expression in the clones was still more than 10-fold that seen in bulk cells transduced with the original Δ3-5 vector. Therefore, the S35 gene unit should be much more effective in a gene therapy setting in which bulk cells are removed, transduced and then reintroduced back into a patient.

Example 30: Stability

Finally, the bulk S35-transduced line, resistant to G418, was propogated for a period of 3 months (in the absence of G418) to determine if ribozyme expression was stable over extended periods of time. This situation mimicks that found in the clinic in which bulk cells are transduced and then reintroduced into the patient and allowed to propogate. There was a modest 30% reduction of ribozyme expression after 3 months. This difference probably arose from cells with varying amount of ribozyme expression and exhibiting different growth rates in the culture becoming slightly more prevalent in the culture. However, ribozyme expression is apparently stable for at least this period of time.

Example 31: Design and construction of TRZ-tRNA Chimera

A transcription unit, termed **TRZ**, is designed that contains the **S35** motif (Figure 52). A desired RNA (e.g. ribozyme) can be inserted into the indicated region of TRZ tRNA chimera. This construct might provide additional stability to the desired RNA. TRZ-A and TRZ-B are non-limiting examples of the TRZ-tRNA chimera.

Referring to Fig. 53-54, a hammerhead ribozyme targeted to site I (HHITRZ-A; Fig. 53) and a hairpin ribozyme (HPITRZ-A; Fig. 54), also targeted to site I, is cloned individually into the indicated region of TRZ tRNA chimera. The resulting ribozyme trancripts retain full RNA cleavage activity (see for example Fig. 55). Applicant has shown that efficient

expression of these TRZ tRNA chimera can be achieved in mammalian cells.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated region of TRZ-tRNA chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in Figures 40-47 and 50 - 54 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Example 32: Ribozyme expression in T cell lines

Ribozyme expression in T cell lines stably-transduced with either a retroviral-based or an Adeno-associated virus (AAV)-based ribozyme expression vector (Figure 56). The human T cell lines MT2 and CEM were transduced with either retroviral or AAV vectors encoding a neomycin slelctable marker and a ribozyme (S35/HHI) expressed from pol III met_i tRNA-driven promoter. Cells stably-transduced with the vectors were selectivelyt expanded medium containing the neomycin antibiotic derivative, G418 (0.7 mg/ml). Ribozyme expression in the stable cell lines was then alalyzed by Northern analysis. The probe used to detect ribozyme transcripts also cross-hybridized with human met_i tRNA sequences. Refering to Figure 56, S35/HHI RNA accumulates to significant levels in MT2 and CEM cells when transduced with either the retrovirus or the AAV vector.

These are meant to be non-limiting examples, those skilled in the art will recognize that other vectors such as adenovirus vector (Figure 57), plasmid DNA vector, alpha virus vectors and the other derivatives there of, can be readily generated to deliver the desired RNA, using techniques known in the art and are within the scope of this invention. Additionally, the transcription units can be expressed individually or in multiples using pol II and/or pol III promoters.

References cited herein, as well as Draper WO 93/23569, 94/02495, 94/06331, Sullenger WO 93/12657, Thompson WO 93/04573, and Sullivan

WO 94/04609, and 93/11253 describe methods for use of vectors decribed herein, and are incorporated by reference herein. In particular these vectors are useful for administration of antisense and decoy RNA molecules.

Example 33: Ligated Ribozymes are catalytically active

The ability of ribozymes generated by ligation methods, described in Draper et al., PCT WO 93/23569, to cleave target RNA was tested on either matched substrate RNA (Fig. 58) or long (622 nt) RNA (Fig. 59, 60 and 61).

Matched substrate RNAs were chemically synthesized using solid-phase RNA synthesis chemistry (Scaringe et al., 1990 Nucleic Acids Res. 18, 5433-5441). Substrate RNA was 5' end-labeled using [γ-^{32}P] ATP and polynucleotide kinase (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). Ribozyme reactions were carried out under ribozyme excess conditions (k_{cat}/K_M; Herschlag and Cech, 1990 Biochemistry 29, 10159-10171). Briefly, ribozyme and substrate RNA were denatured and renatured separately by heating to 90°C and snap cooling on ice for 10 min in a buffer containing 50 mM Tris. HCl pH 7.5 and 10 mM $MgCl_2$. Cleavage reaction was initiated by mixing the ribozyme with the substrate at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was stopped by mixing with equal volume of formamide gel loading buffer (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). The samples were resolved on 20 % polyacrylamide-urea gel. Refering to Fig. 58, -ΔG refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 Supra). RPI A is a HH ribozyme with 6/6 binding arms. This ribozyme was synthesized chemically either as a one piece ribozyme or was synthesized in two fragments followed by ligation to generate a one piece ribozyme. The k_{cat}/K_M values for the two ribozymes were comparable.

A template containing T7 RNA polymerase promoter upstream of 622 nt long target sequence, was PCR amplified from a DNA clone. The target RNA (containing HH ribozyme cleavage sites B, C and D) was transcribed from this PCR amplified template using T7 RNA polymerase. The transcript was internally labeled during transcription by including [α-^{32}P] CTP as one of the four ribonucleotide triphosphates. The transcription mixture was

treated with DNase-1, following transcription at 37°C for 2 hours, to digest away the DNA template used in the transcription. RNA was precipitated with Isopropanol and the pellet was washed two times with 70% ethanol to get rid of salt and nucleotides used in the transcription reaction. RNA is resuspended in DEPC-treated water and stored at 4°C. Ribozyme cleavage reactions were carried out under ribozyme excess (k_{cat}/K_M) conditions [Herschlag and Cech 1990 supra]. Briefly, 1000 nM ribozyme and 10 nM internally labeled target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris.HCl, pH 7.5 and 10 mM $MgCl_2$. The RNAs were renatured by cooling to 37°C for 10-20 min. Cleavage reaction was initiated by mixing the ribozyme and target RNA at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was quenched by adding equal volume of stop buffer. The samples were resolved on a sequencing gel.

Example 34: Hammerhead ribozymes with ≥ 2 base-paired stem II are catalytically active

To decrease the cost of chemical synthesis of RNA, applicant was interested in determining whether the length of stem II region of a typical hammerhead ribozyme (≥ 4 bp stem II) can be shortened without decreasing the catalytic efficiency of the HH ribozyme. The length of stem II was systematically shortened by one base-pair at a time. HH ribozymes with three and two base-paired stem II were chemically synthesized using solid-phase RNA phosphoramidite chemistry (Scaringe et al., 1990 supra).

Matched and long substrate RNAs were synthesized and ribozyme assays were carried out as described in example 33. Referring to figures , 62, 63 and 64, data shows that shortening stem II of a hammerhead ribozyme does not significantly alter the catalytic efficiency. It is applicant's opinion that hammerhead ribozymes with ≥ 2 base-paired stem II region are catalytically active.

Example 35: Synthesis of catalytically active hairpin ribozymes

RNA molecules were chemically synthesized having the nucleotide base sequence shown in Fig. 65 for both the 5' and 3' fragments. The 3' fragments are phosphorylated and ligated to the 5' fragment essentially as described in example 37. As is evident from the Figure 65, the 3' and 5' fragments can hybridize together at helix 4 and are covalently linked via

GAAA sequence. When this structure hybridizes to a substrate, a ribozym •substrate complex structure is formed. While helix 4 is shown as 3 base pairs it may be formed with only 1 or 2 base pairs.

40 nM mixtures of ligated ribozymes were incubated with 1-5 nM 5' end-labeled matched substrates (chemically synthesized by solid-phase synthesis using RNA phosphoramidite chemistry) for different times in 50 mM Tris/HCl pH 7.5, 10 mM $MgCl_2$ and shown to cleave the substrate efficiently (Fig.66).

The target and the ribozyme sequences shown in Fig. 62 and 65 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using other sequences and techniques generally known in the art.

V. Constructs of Hairpin Ribozymes

There follows an improved trans-cleaving hairpin ribozyme in which a new helix (*i.e.*, a sequence able to form a double-stranded region with another single-stranded nucleic acid) is provided in the ribozyme to base-pair with a 5' region of a separate substrate nucleic acid. This helix is provided at the 3' end of the ribozyme after helix 3 as shown in Figure 3. In addition, at least two extra bases may be provided in helix 2 and a portion of the substrate corresponding to helix 2 may be either directly linked to the 5' portion able to hydrogen bond to the 3' end of the hairpin or may have a linker of atleast one base. By trans-cleaving is meant that the ribozyme is able to act in *trans* to cleave another RNA molecule which is not covalently linked to the ribozyme itself. Thus, the ribozyme is not able to act on itself in an intramolecular cleavage reaction.

By "base-pair" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of interactions.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) has several advantages. These include improved stability of the ribozyme-target complex *in vivo* . In addition, an increase in the recognition sequence of the hairpin ribozyme improves the specificity of the ribozyme. This also makes possible the targeting of potential hairpin

ribozyme sites that would otherwise be inaccessible due to neighboring secondary structure.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) enhances *trans*-ligation reaction catalyzed by the ribozyme. *Trans*-ligation reactions catalyzed by the regular hairpin ribozyme (4 bp helix 2) is very inefficient (Komatsu *et al.*, 1993 *Nucleic Acids Res.* 21, 185). This is attributed to weak base-pairing interactions between substrate RNAs and the ribozyme. By increasing the length of helix 2 (with or without helix 5) the rate of ligation (*in vitro* and *in vivo*) can be enhanced several fold.

Results of experiments suggest that the length of H2 can be 6 bp without significantly reducing the activity of the hairpin ribozyme. The H2 arm length variation does not appear to be sequence dependent. HP ribozymes with 6 bp H2 have been designed against five different target RNAs and all five ribozymes efficiently cleaved their cognate target RNA. Additionally, two of these ribozymes were able to successfully inhibit gene expression (e.g., TNF-α) in mammalian cells. Results of these experiments are shown below.

HP ribozymes with 7 and 8 bp H2 are also capable of cleaving target RNA in a sequence-specific manner, however, the rate of the cleavage reaction is lower than those catalyzed by HP ribozymes with 6 bp H2.

Example 36: 4 and 6 base pair H2

Referring to Figures 67-72, HP ribozymes were synthesized as described above and tested for activity. Surprisingly, those with 6 base pairs in H2 were still as active as those with 4 base pairs.

VI. Chemical Modification

Oligonucleotides with 5'-C-alkyl Group

The introduction of an alkyl group at the 5'-position of a nucleoside or nucleotide sugar introduces an additional center of chirality into the sugar moiety. Referring to Fig. 75, the general structures of 5'-*C*-alkylnucleotides belonging to the D-allose, **2**, and L-talose, **3**, sugar families are shown. The family names are derived from the known sugars D-allose and L-talose ($R_1 = CH_3$ in **2** and **3** in Figure 75). Useful specific D-allose and L-talose

nucleotide derivatives are shown in Figure 76, **29-32** and Figure 77, **58-61** respectively.

This invention relates to the use of 5'-*C*-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 5'-*C*-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 5'-*C*-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 5'-*C*-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 5'-*C*-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 5'-*C*-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 5'-*C*-alkylnucleosides, that is a nucleotide base having at the 5'-position on the sugar molecule an alkyl moiety. In a related aspect, the invention also features 5'-*C*-alkylnucleotides, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably

includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above. In preferred embodiments, the sugar of the nucleoside or nucleotide is in an optically pure form, as the talose or allose sugar.

Examples of various alkyl groups useful in this invention are shown in Figure 75, where each R_1 group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. Wh n substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above. Carbocyclic aryl groups are groups wherein the ring

atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 5'-*C*-alkylnucleotides; *e.g.* enzymatic nucleic acids having a 5'-*C*-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 5'-position an alkyl group. In other related aspects, the invention features 5'-*C*-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 5'-*C*-alkyl derivatives of this invention provide enhanced stability to the oligonulceotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features a method for conversion of a protected allo sugar to a protected talo sugar. In the method, the protected allo sugar is contacted with triphenyl phosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid under inversion causing conditions to provide the protected talo sugar. While one example of such conditions is provided below, those in the art will recognize other such conditions. Applicant has found that such conversion allows for ready synthesis of all types of nucleotide bases as exemplified in the figures.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particulary useful for enzymatic RNA molecules. Thus, below is provided examples of such

molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 5'-*C*-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides shown in Figure 75 are possible.

The following are non-limiting examples showing the synthesis of nucleic acids using 5'-*C*-alkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 37: Synthesis of Hammerhead Ribozymes Containing 5'-*C*-Alkyl-nucleotides & Other Modified Nucleotides

The method of synthesis would follow the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds **26-29** and **56-59**). These 5'-*C*-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 38: Methyl-2,3-*O*-Isopropylidine-6-Deoxy-β-D-allofuranoside (4)

A suspension of L-rhamnose (100 g, 0.55 mol), $CuSO_4$ (120 g) and conc. H_2SO_4 (4.0 mL) in 1.0 L of dry acetone was mixed for 24 h at RT, then filtered. Conc. NH_4OH (5 mL) was added to the filtrate and the newly formed precipitate was filtered. The residue was concentrated *in vacuo*, coevaporated with pyridine (2 x 300 mL), dissolved in pyridine (500 mL) and cooled to 0 °C. A solution of *p*-tolu nesufonylchloride (107 g , 0.56

mmol) in dry DCE (500 mL) was added dropwise over 0.5 h. The reaction mixture was left for 16 h at RT. The reaction was quenched by adding ice-water (0.5 L) and, after mixing for 0.5 h, was extracted with chloroform (0.75 L). The organic layer was washed with H_2O (2 x 500 mL), 10% H_2SO_4 (2 x 300 mL), water (2 x 300 mL), sat. $NaHCO_3$ (2 x 300 mL), brine (2 x 300 mL), dried over $MgSO_4$ and evaporated to dryness. The residue (115 g) was dissolved in dry MeOH (1 L) and treated with NaOMe (23.2 g, 0.42 mmol) in MeOH. The reaction mixture was left for 16 h at 20 °C, neutralized with dry CO_2 and evaporated to dryness. The residue was suspended in chloroform (750 mL), filtered , concentrated to 100 mL and purified by flash chromatography in $CHCl_3$ to yield 45 g (37%) of compound **4**.

Example 39: Methyl-2,3-*O*-Isopropylidine-5-*O*-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (5).

To solution of methylfuranoside 4 (12.5 g 62.2 mmol) and $AgNO_3$ (21.25 g, 125.0 mmol) in dry DMF (300 mL) *t*-butyldiphenylsilyl chloride (22.2 g , 81 mmol) was added dropwise under Ar over 0.5 h. The reaction mixture was stirred for 4 h at RT, diluted with $CHCl_3$ (200 mL), filtered and evaporated to dryness (below 40 °C using a high vacuum oil pump). The residue was dissolved in CH_2Cl_2 (300 mL) washed with sat. $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL), dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography in CH_2Cl_2 to yield 20.0 g (75%) of compound **5**.

Example 40: Methyl-5-*O*-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (6).

Methylfuranoside **5** (13.5 g, 30.6 mmol) was dissolved in CF_3COOH:dioxane:H_2O / 2:1:1 (v/v/v, 200 mL) and stirred at 24 °C for 45 m. The reaction mixture was cooled to -10 °C, neutralized with conc. NH_4OH (140 mL) and extracted with CH_2Cl_2 (500 mL). The organic layer was separated, washed with sat. $NaHCO_3$ (2 x 75 mL), brine (2 x 75 mL), dried over $MgSO_4$ and evaporated to dryness. The product **6** was purified by flash chromatography using a 0-10% MeOH gradient in CH_2Cl_2. Yield 9.0 g (76%).

Example 41: Methyl-2,3-di-*O*-Benzoyl-5-*O*-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranosid (7).

Methylfuranoside 6 (7.0 g, 17.5 mmol) was coevaporated with pyridine (2 x 100 mL) and dissolved in pyridine (100 mL). Benzoyl chloride (5.4 g, 38.5 mmol) was added and the reaction mixture was left at RT for 16 h. Dry EtOH (50 mL) was added and the reaction mixture was evaporated to dryness after 0.5 h. The residue was dissolved in CH_2Cl_2 (300 mL), washed with sat. $NaHCO_3$ (2 x 75 mL), brine (2 x 75 mL) dried over $MgSO_4$ and evaporated to dryness. The product was purified by flash chromatography in CH_2Cl_2 to yield 9.5 g (89%) of compound 7.

Example 42: 1-*O*-Acetyl-2,3-di-*O*-benzoyl-5-*O*-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranose (8).

Dibenzoate 7 (4.7 g, 7.7 mmol) was dissolved in a mixture of AcOH (10.0 mL), Ac_2O (20.0 mL) and EtOAc (30 mL) and the reaction mixture was cooled 0 °C. 98% H_2SO_4 (0.15 mL) was then added. The reaction mixture was kept at 0 °C for 16 h, and then poured into a cold 1:1 mixture of sat. $NaHCO_3$ and EtOAc (150 mL). After 0.5 h of vigorous stirring the organic phase was separated, washed with brine (2 x 75 mL), dried over $MgSO_4$, evaporated to dryness and coevaporated with toluene (2 x 50 mL). The product was purified by flash chromatography using a gradient of 0-5% MeOH in CH_2Cl_2. Yield: 4.0 g (82% as a mixture of α and β isomers).

Example 43: 1-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β–D-Allofuranosyl)uracil (9).

Uracil (1.44 g, 11.5 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT, evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (6.36 g, 10.0 mmol) in dry CH_3CN (100 mL), followed by $CF_3SO_3SiMe_3$ (2.8 g, 12.6 mmol). The reaction mixture was kept at 24 °C for 16 h, concentrated to 1/3 of its original volume, diluted with 100 mL of CH_2Cl_2 and extracted with sat. $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL) dried over $MgSO_4$, and evaporated to dryness. The product 9 was purified by flash chromatography using a gradient of 0-5% MeOH in CH_2Cl_2. Yield: 5.7 g (80%).

Example 44: *N*4-Benzoyl-1-(2',3'-Di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-ß-D-Allofuranosyl)Cytosine (10).

*N*4-benzoylcytosine (1.84 g, 8.56 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of of acetates **8** (3.6 g, 5.6 mmol) in dry CH_3CN (100 mL), followed by $CF_3SO_3SiMe_3$ (4.76 g, 21.4 mmol). The reaction mixture was boiled under reflux for 5 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH_2Cl_2 (100 mL) and extracted with sat. $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL) dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography using a gradient of 0-5% MeOH in CH_2Cl_2 yielded 1.8 g (55%) of compound **10**.

Example 45: *N*6-Benzoyl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-ß-D-Allofuranosyl)adenine (11).

*N*6-benzoyladenine (2.86 g, 11.86 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (7 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of of acetates **8** (3.6 g, 5.6 mmol) in dry CH_3CN (100 mL) followed by $CF_3SO_3SiMe_3$ (6.59 g, 29.7 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH_2Cl_2 (100 mL) and extracted with sat. $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL) dried over $MgSO_4$ and evaporated to dryness. The product **11** was purified by flash chromatography using a gradient of 0-5% MeOH in CH_2Cl_2. Yield: 2.7 g (60%).

Example 46: *N*2-Isobutyryl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-ß-D-Allofuranosyl)guanine (12).

*N*2-Isobutyrylguanine (1.47 g , 11.2 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (6 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a

solution of of acetates **8** (3.4 g, 5.3 mmol) in dry CH_3CN (100 mL) followed by $CF_3SO_3SiMe_3$ (6.22 g, 28.0 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volum , diluted with CH_2Cl_2 (100 mL) and extracted with sat. $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL) dried over $MgSO_4$ and evaporated to dryness. The product **12** was purified by flash chromatography using a gradient of 0-2% MeOH in CH_2Cl_2. Yield: 2.1g (54%).

<u>Example 47: *N*⁶-Benzoyl-9-(2',3'-di-*O*-benzoyl-6'-Deoxy-β-D-Allofuranosyl)adenine (15).</u>

Nucleoside **11** (1.65 g, 2.0 mmol) was dissolved in THF (50 mL) and a 1 M solution of TBAF in THF (4 mL) was added. The reaction mixture was kept at RT for 4 h, evaporated to dryness and the product purified by flash chromatography using a gradient of 0-5% MeOH in CH_2Cl_2 to yield 1.0 g (85%) of compound **15**.

<u>Example 48: *N*⁶-Benzoyl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)-adenine (19).</u>

Nucleoside **15** (0.55 g, 0.92 mmol) was dissolved in dry CH_2Cl_2 (50 mL). $AgNO_3$ (0.34 g, 2.0 mmol), dimethoxytrityl chloride (0.68 g, 2.0 mmol) and sym-collidine (0.48 g) were added under Ar. The reaction mixture was stirred for 2h, diluted with CH_2Cl_2 (100 mL), filtered, evaporated to dryness and coevaporated with toluene (2 x 50 mL). Purification by flash chromatography using a gradient of 0-5% MeOH in CH_2Cl_2 yielded 0.8 g (97%) of compound **19**.

<u>Example 49: *N*⁶-Benzoyl-9-(-5'-*O*-Dimethoxytrityl-6'-Deoxy-β–D-Allofuranosyl)adenine (23).</u>

Nucleoside **19** (1.8 g, 2 mmol) was dissolved in dioxane (50 mL), cooled to 0 °C and 2 M NaOH (50 mL) was added. The reaction mixture was kept at 0 °C for 45 m, neutralized with Dowex 50 (Pyr^+ form), filtered and the resin was washed with MeOH (2 x 50 mL). The filtrate was then evaporated to dryness. Purification by flash chromatography using a gradient of 0-10% MeOH in CH_2Cl_2 yielded 1.1 g (80%) of **23**.

Example 50: N^6-Benzoyl-9-(-5'-*O*-Dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyl-6'-Deoxy-β–D-Allofuranosyl)adenine (27).

Nucleoside **23** (1.2 g, 1.8 mmol) was dissolved in dry THF (50 mL). Pyridine (0.50 g, 8 mmol) and $AgNO_3$ (0.4 g, 2.3 mmol) were added. After the $AgNO_3$ dissolved (1.5 h), *t*-butyldimethylsilyl chloride (0.35 g , 2.3 mmol) was added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), filtered into sat. $NaHCO_3$ (50 mL), extracted, the organic layer washed with brine (2 x 50 mL), dried over $MgSO_4$ and evaporated to dryness. The product **27** was purified by flash chromatography using a hexanes:EtOAc / 7:3 gradient. Yield: 0.7 g (50%).

Example 51: N^6-Benzoyl-9-(-5'-*O*-Dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyl-6'-Deoxy-β–D-Allofuranosyl)adenine-3'-(2-Cyanoethyl *N,N*-diisopropyl-phosphoramidite) (31).

Standard phosphitylation of **27** according to Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 yielded phosphoramidite **31** in 73% yield.

Example 52: Methyl-5-*O*-*p*-Nitrobenzoyl-2,3-*O*-Isopropylidine-6-deoxy-β-L-Tallofuranoside (5)

Methylfuranoside **4** (3.1 g 14.2 mmol) was dissolved in dry dioxane (200 mL), *p*-nitrobenzoic acid (10.0 g, 60 mmol) and triphenylphosphine (15.74 g, 60.0 mmol) were added followed by DEAD (10.45 g, 60.0 mmol). The reaction mixture was left at RT for 16 h, EtOH (5 mL) was added, and after 0.5 h the reaction mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (300 mL) washed with sat. $NaHCO_3$ (2 x 75 mL), brine (2 x 75 mL) dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography using a hexanes:EtOAc / 9:1 gradient yielded 4.1 g (78%) of compound **33**. Subsequent debenzoylation (NaOMe/MeOH) and silylation (see preparation of **5**) led to L-talofuranoside **34** which was converted to phosphoramidites **58-61** using the same methodology as described above for the preparation of the phosphoramidites of the D-allo-isomers **29-32**.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage

or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedur . Administration of such oligonucleotides is by standard procedure. See Sullivan et al., PCT WO 94/ 02595.

The ribozymes and the target RNA containing site O were synthesized, deprotected and purified as described above. RNA cleavage assay was carried our at 37°C in the presence of 10 mM $MgCl_2$ as described above.

Applicant has substituted 5'-C-Me-L-talo nucleotides at positions A6, A9, A9 + G10, C11.1 and C11.1 + G10, as shown in Figure 78 (**HH-O1 to HH-05**). ***HH-O 1,2,4*** and ***5*** showed almost wild type activity (Figure 79). However, ***HH-03*** demonstrated low catalytic activity. Ribozymes ***HH-01, 2, 3, 4 and 5*** are also extremely resistant to degradation by human serum nucleases.

<u>Oligonucleotides with 2'-Deoxy-2'-Alkylnucleotide</u>

This invention uses 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker *et al.* applicant has found that such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair

forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less cons_quence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 81, where each R group is any alkyl. The term "alkyl" does not include alkoxy groups which have an "-*O*-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); *e.g.* enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonulceotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall

activity *in vivo*. Below are provided assays to determine which such molecules are us ful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 80 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 80, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra.*

Figure 80 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 80 the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-*C*-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 81 are possible, and were indeed synthesized, the basic structure composed of promarily 2'-*O*-Me nucleotides weth selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* **1992**, 31, 5005-5009 and Paolella *et al.*, *EMBO J.* **1992**, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 80 and Table 45 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at lease 1/10 of the wild-type catalytic activity. From Table 45, all 2'-modified ribozymes showed very larg and significant increases in stability in human serum (shown) and in the other fluids describ d below (Example 55, data not shown). The order of most agressive nuclease activity was fetal bovine

serum, > human serum >human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio ß was calculated (Table 45). This ß value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in ß indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 81 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 86 compound **37** may be used as a general intermediate to prepare derivatized 2'*C*-alkyl phosphoramidites, where X is CH_3, or an alkyl, or other group described above.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-*C*-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance.

Example 53: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkylnucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds **10**, **12**, **17**, **22**, **31**, **18**, **26**, **32**, **36** and **38**). Other 2'-modified phosphoramidites were prepared according to: **3** & **4**, Eckstein *et al. International Publication* No. WO 92/07065; and **5** Kois *et al. Nucleosides & Nucleotides* **1993**, *12*, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 80. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense

oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 54: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM $MgCl_2$. Reactions were initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 55: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots w re withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme *vs* the time of incubation and extrapolation from the graph.

Example 56: 3',5'-*O*-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-*O*-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid*

Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylaminopyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of **7**.

Example 57: 3',5'-*O*-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-*C*-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)-2'-*O*-phenoxythiocarbonyl-uridine, **7**, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product **8** purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 58: 5'-*O*-Dimethoxytrityl-2'-*C*-Allyl-Uridine (9)

A solution of **8** (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated, dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of **9** as a white foam.

Example 59: 5'-*O*-Dimethoxytrityl-2'-*C*-Allyl-Uridine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (10)

5'-*O*-Dimethoxytrityl-2'-*C*-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. *N,N*-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 60: 3',5'-*O*-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-*C*-Allyl-*N*4-Acetyl-Cytidine (11)

Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)-2'-*C*-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH_4OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as a white foam.

Example 61: 5'-*O*-Dimethoxytrityl-2'-*C*-Allyl-*N*4-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 62: 5'-*O*-Dimethoxytrityl-2'-*C*-allyl-*N*4-Acetyl-Cytidine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (12)

2'-*O*-Dimethoxytrityl-2'-*C*-allyl-N^4-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. *N,N*-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 63: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, *40*, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 1991, *34*, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH_2Cl_2.

Example 64: 5'-*O*-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$, water and brine. The organic extracts were dried over $MgSO_4$, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexan s as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 65: 5'-*O*-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH_2Cl_2 (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH_2Cl_2: MeOH / 15:1)

Example 66: 2'-Deoxy-2'-Difluoromethylene-3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **14** (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 67: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH_2Cl_2.

Example 68: 5'-*O*-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolv d in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture

was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$, water and brine. The organic extracts were dried over $MgSO_4$, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-*O*-DMT-2'-deoxy-2'-difluoromethylene-uridine **16** (1.05 g, 1.8 mmol, 45%).

Example 69: 5'-*O*-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (**18**)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH_2Cl_2 (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH_2Cl_2: MeOH / 15:1).

Example 70: 2'-Deoxy-2'-Methylene-3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-4-*N*-Acetyl-Cytidine **20**

Triethylamine (4.8 mL, 34 mmol) was added to a solution of $POCl_3$ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 x 100 mL) and washed with 5% $NaHCO_3$ (1 x 100 mL). The organic extracts were dried over Na_2SO_4 concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq. ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. $NaHCO_3$ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 x 100 mL) and washed with 5% $NaHCO_3$ (1 x 100 mL). The

organic extracts were dried over Na_2SO_4, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4-*N*-acetyl-cytidine **20** (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

Example 71: 1-(2'-Deoxy-2'-Methylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetyl-Cytosine **21**

2'-Deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4-*N*-acetyl-cytidine **20** (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-*N*-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH_2Cl_2. 2'-Deoxy-2'-methylene-4-*N*-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over $MgSO_4$, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **21** (0.88 g, 1.5 mmol, 75%).

Example 72: 1-(2'-Deoxy-2'-Methylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetyl-Cytosine 3'-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite) **(22)**

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-acetyl-cytosine **21** (0.88 g, 1.5 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product **22** (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH_2Cl_2:MeOH / 20:1).

Example 73: 2'-Deoxy-2'-Difluoromethylene-3',5'-*O*-(Tetraisopropyl disiloxane-1,3-diyl)-4-*N*-Acetyl-Cytidine (24)

Et_3N (6.9 mL, 50 mmol) was added to a solution of $POCl_3$ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **23** ([described in example 14] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 x 100 mL) and washed with 5% $NaHCO_3$ (1 x 100 mL). The organic extracts were dried over Na_2SO_4 concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. $NaHCO_3$ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 x 100 mL) and washed with 5% $NaHCO_3$ (1 x 100 mL). The organic extracts were dried over Na_2SO_4, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4-*N*-acetyl-cytidine **24** (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 74: 1-(2'-Deoxy-2'-Difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4-*N*-acetyl-cytidine **24** (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-*N*-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH_2Cl_2. 2'-Deoxy-2'-difluoromethylene-4-*N*-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over $MgSO_4$, concentrated *in*

vacuo and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **25** (1.2 g, 1.9 mmol, 68%).

Example 75: 1-(2'-Deoxy-2'-Difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetylcytosine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**26**)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-acetylcytosine **25** (0.6 g, 0.97 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product **26**, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH_2Cl_2:MeOH / 20:1).

Example 76: 2'-Keto-3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (**28**)

Acetic anhydride (4.6 mL) was added to a solution of 3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (Brown,J.; Christodolou, C.; Jones,S.; Modak,A.; Reese,C.; Sibanda,S.; Ubasawa A. *J. Chem .Soc. Perkin Trans. I* **1989**, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine **28** (4.8 g, 7.2 mmol, 78%).

Example 77: 2'-Deoxy-2'-methylene-3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (**29**)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g,17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine

28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH_2Cl_2 (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H_2O (20 mL), 5% aqueous $NaHCO_3$ (20 mL), H_2O to neutrality, and brine (10 mL). After drying (Na_2SO_4), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine **29** (3.86 g, 5.8 mmol, 79%).

Example 78: 2'-Deoxy-2'-Methylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-methylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH_2Cl_2.

Example 79: 5'-*O*-DMT-2'-Deoxy-2'-Methylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (29)

2'-Deoxy-2'-methylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$, water and brine. The organic extracts were dried over $MgSO_4$, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield **29** (0.81 g, 1.1 mmol, 62%).

Example 80: 5'-*O*-DMT-2'-Deoxy-2'-Methylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine **29** dissolved in dry CH_2Cl_2 (15 mL) was placed

in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). R_f 0.45 (CH_2Cl_2: MeOH / 20:1)

Example 81: 2'-Deoxy-2'-Difluoromethylene-3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine **28** (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 82: 2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH_2Cl_2.

Example 83: 5'-*O*-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in

pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$, water and brine. The organic extracts were dried over $MgSO_4$, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield **30** (2.6 g, 3.41 mmol, 69%).

Example 84: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (**32**)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine **30** (2.6 g, 3.4 mmol) dissolved in dry CH_2Cl_2 (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). **32** (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH_2Cl_2: MeOH / 15:1).

Example 85: 2'-Deoxy-2'-Methoxycarbonylmethylidine-3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine (**33**)

Methyl(triphenylphosphoranylidine)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine **14** in CH_2Cl_2 under argon. The mixture was left to stir at RT for 30 h. CH_2Cl_2 (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H_2O (20 mL), 5% aq. $NaHCO_3$ (20 mL), H_2O to neutrality, and brine (10 mL). After drying (Na_2SO_4), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidine-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine **33** (5.8 g, 10.8 mmol, 67.5%).

Example 86: 2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (34)

$Et_3N \cdot 3HF$ (3 mL) was added to a solution of 2'-deoxy-2'-methoxy-carboxylmethylidine-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH_2Cl_2 (20 mL) and Et_3N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidine-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH_2Cl_2 / 4:1.

Example 87: 5'-*O*-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (35)

2'-Deoxy-2'-methoxycarbonylmethylidine-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$, water and brine. The organic extracts were dried over $MgSO_4$, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH_2Cl_2 as an eluant to yield 5'-*O*-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine **35** (2.03 g, 3.46 mmol, 86%).

Example 88: 5'-*O*-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidine-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-*O*-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.44 (CH_2Cl_2:M OH / 9.5:0.5).

Example 89: 2'-Deoxy-2'-Carboxymethylidine-3',5'-*O*-(Tetraisopropyldi-siloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidine-3',5'-*O*-(tetraisopropyldi-siloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidine-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH_2Cl_2.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* *PCT WO* 94/02595.

Oligonucleotides with 3' and/or 5' Dihalophosphonate

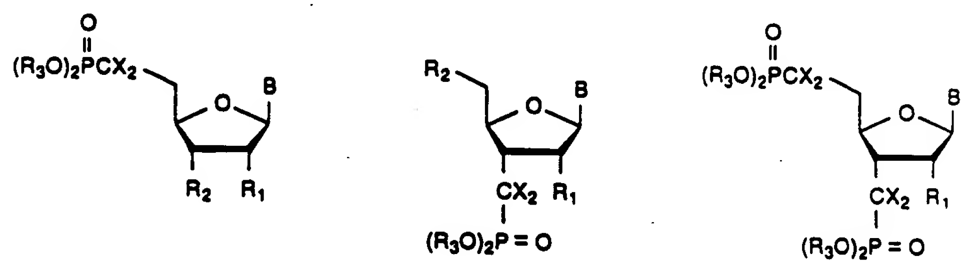
This invention synthesis and uses 3' and/or 5' dihalophosphonate-, e.g., 3' or 5'-CF_2-phosphonate-, substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of an enzymatic or antisense molecule.

As the term is used in this application, 5'- and/or 3'-dihalophosphonate nucleotide containing ribozymes, deoxyribozymes (see Usman et al., PCT/US94/11649, incorporated by reference herein), and chimeras of nucleotides, are catalytic nucleic molecules that contain 5'- and/or 3'-dihalophosphonate nucleotide components replacing, but not limited to, double-stranded stems, single-stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA or DNA transcript. This invention concerns

nucleic acids formed of standard nucleotides or modified nucleotides, which also contain at least one 5'-dihalophosphonate and/or one 3'-dihalophosphonate group.

The synthesis of 1-*O*-Ac-2,3-di-*O*-Bz-D-ribofuranose 5-d-5+dihalomethylphosphonate in three steps from 1-*O*-methyl-2,3-*O*-isopropylidene-ß-D-ribofuranose 5-deoxy-5-dihalomethylphosphonate is described (e.g., for the difluoro, in Figure 87). Condensation of this suitably derivatized sugar with silylated pyrimidines and purines affords novel nucleoside 5'-deoxy-5'-dihalomethylphosphonates. These intermediates may be incorporated into catalytic or antisense nucleic acids by either chemical (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into suitably protected phosphoramidites **12a** or solid supports **12b**, e.g., Figure 88) or enzymatic means (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into their triphosphates, e.g., **14** Figure 89, for T7 transcription).

Thus, in one aspect the invention features 5' and/or 3'-dihalonucleotides and nucleic acids containing such 5' and/or 3'-dihalonucleotides. The general structure of such molecules is shown below.

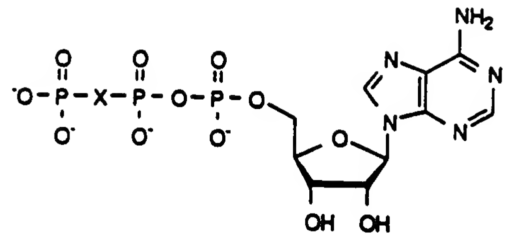


where R_1 is H, OH, or R, where R is a hydroxyl protecting group, e.g., acyl, alkysilyl, or carbonate; each R_2 is separately H, OH, or R; each R_3 is separately a phosphate protecting group, e.g., methyl, ethyl, cyanoethyl, p-nitrophenyl, or chlorophenyl; each X is separately any halogen; and each B is any nucleotide base.

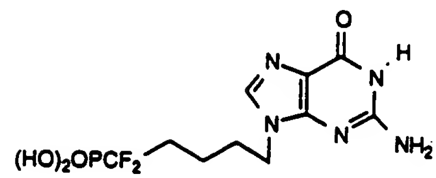
The invention in particular features nucleic acid molecules having such modified nucleotides and enzymatic activity. In a related aspect the invention features a method for synthesis of such nucleoside 5'-deoxy-5'-dihalo and/or 3'-deoxy-3'-dihalophosphonates by condensing a

dihalophosphonate-containing sugar with a pyrimidine or a purine under conditions suitable to form a nucleoside 5'-deoxy-5'-dihalophosphonate and/or a 3'-deoxy-3'-dihalophosphonate.

Phosphonic acids may exhibit important biological properties because of their similarity to phosphates (Engel, *Chem. Rev.* 1977, 77, 349-367). Blackburn and Kent (*J. Chem. Soc., Perkin Trans.* 1986, 913-917) indicate that based on electronic and steric considerations _-fluoro and _,_-difluoromethylphosphonates might mimic phosphate esters better than the corresponding phosphonates. Analogues of pyro- and triphosphates **1**, where the bridging oxygen atoms are replaced by a difluoromethylene group, have been employed as substrates in enzymatic processes (Blackburn *et al.*, *Nucleosides & Nucleotides* 1985, 4, 165-167; Blackburn *et al.*, *Chem. Scr.* 1986, 26, 21-24). 9-(5,5-Difluoro-5-phosphonopentyl)guanine (**2**) has been utilized as a multisubstrate analogue inhibitor of purine nucleoside phosphorylase (Halazy *et al.*, *J. Am. Chem. Soc.* 1991, 113, 315-317). Oligonucleotides containing methylene groups in place of phosphodiester 5'-oxygens are resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5'-oxygen (Breaker *et al.*, *Biochemistry* 1993, 32, 9125-9128), but can still form stable complexes with complementary sequences. Heinemann *et al.* (*Nucleic Acids Res.* 1991, 19, 427-433) found that a single 3'-methylenephosphonate linkage had a minor influence on the conformation of a DNA octamer double helix.



1



2

$(ETO)_2POCF_2Li$

3

One common synthetic approach to α,α-difluoro-alkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate (**3**) (Obayashi *et al.*, *Tetrahedron Lett.* 1982, 23, 2323-2326). However, our attempts to synthesize nucleoside 5'-deoxy-5'-difluoro-methylphosphonates from 5'-deoxy-5'-iodonucleosides using **3** were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with **3**, according to the procedure of Martin *et al.* (Martin *et al.*, *Tetrahedron Lett.* 1992, 33, 1839-1842), led to a complex mixture of products. Recently, the synthesis of sugar α,α-difluoroalkylphosphonates from primary sugar triflates using **3** was described (Berkowitz *et al.*, *J. Org. Chem.* 1993, 58, 6174-6176). Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.

The following are non-limiting exampl s showing the synthesis of nucleoside 5'-deoxy-5'-difluoromethyl-phosphonates. Those in the art will recognize that equivalent methods can be readily devised based upon

these examples. These examples demonstrate that it is possible to achieve synthesis of 5'-deoxy-5'-difluoro derivatives in good yield and thus guide those in the art to such equivalent methods. The examples also indicate utility of such synthesis to provide useful oligonucleotides as described above.

Those in the art will recognize that useful modified enzymatic nucleic acids can now be designed, much as described by Draper et al., PCT/US94/13129 hereby incorporated by reference herein (including drawings).

Example 90: Synthesis of Nucleoside 5'-Deoxy-5'-difluoromethylphosphonates

Referring to Fig. 87, we synthesized a suitable glycosylating agent from the known D-ribose α,α-difluoromethylphosphonate (4) (Martin *et al.*, *Tetrahedron Lett.* 1992, 33, 1839-1842) which served as a key intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

Methyl 2,3-*O*-isopropylidene-β-D-ribofuranose a,a-difluoromethylphosphonate (4) was synthesized from the 5-aldehyde according to the procedure of Martin *et al.* (*Tetrahedron Lett.* **1992**, 33, 1839-1842) (Figure 87). Removal of the isopropylidene group was accomplished under mild conditions (I_2-MeOH, reflux, 18 h (Szarek *et al.*, *Tetrahedron Lett.* **1986**, 27, 3827) or Dowex 50 WX8 (H^+), MeOH, RT (about 20-25°C), 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-di-*O*-benzoyl derivative, which was subjected to mild acetolysis conditions (Walczak *et al.*, *Synthesis*, 1993, 790-792) (Ac_2O, AcOH, H_2SO_4, EtOAc, 0°C. The desired 1-*O*-acetyl-2,3-di-*O*-benzoyl-D-ribofuranose difluoromethylphosphonate (5) was obtained in quantitative yield as an anomeric mixture. These derivatives were used for selective glycosylation of silylated uracil and N^4-acetylcytosine under Vorbrüggen conditions (Vorbrüggen, *Nucleoside Analogs. Chemistry, Biology and Medical Applications, NATO ASI Series A*, 26, Plenum Press, New York, London, 1980; pp. 35-69. The use of $F_3CSO_2OSi(CH_3)_3$ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 1-ethyluracil or 9-ethyladenine byproducts: Podyukova, *et al.*, *Tetrahedron*

Lett. 1987, 28, 3623-3626 and refer nces cited therein) ($SnCl_4$ as a catalyst, boiling acetonitrile) to yield β-nucleosides (62% **6a**, 75% **6b**). Glycosylation of silylated N^6-benzoyladenine under the same conditions yielded a mixture of N-9 isomer **6c** and N-7 isomer **7** in 34% and 15% yield, respectively. The above nucleotides were successfully deprotected using trimethylsilylbromide for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates **8** were finally purified on a DEAE Sephadex A-25 (HCO_3^-) column using a 0.01-0.25 M TEAB gradient for elution and obtained as their sodium salts (82% **8a**; 87% **8b**; 82% **8c**).

Selected analytical data: ^{31}P-NMR (^{31}P) and ^1H-NMR (^1H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was $CDCl_3$ unless otherwise noted. **5:** ^1H δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, αH1), 6.42 (s, βH1), 5.74 (d, $J_{2,3}$ 4.9, βH2), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, βH3), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, αH3), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, αH2), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{P,F}$ 105.2), 7.67 (t, $J_{P,F}$ 106.5). **6a:** ^1H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',F}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{H,F}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{P,F}$ 104.0). **8c:** ^{31}P (*vs* DSS) (D_2O) δ 5.71 (t, $J_{P,F}$ 87.9).

Compound **7** was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

Example 91:Synthesis of Nucleic Acids Containing Modified Nucleotide Containing Cores

.. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in Scaringe *et al.*, *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (Figure 88 and Janda *et al.*, *Science* **1989**, 244:437-440.). These

nucleoside 5'-deoxy-5'-difluoromethylphosphonates may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 introns, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 92: Synthesis of Modified Triphosphate

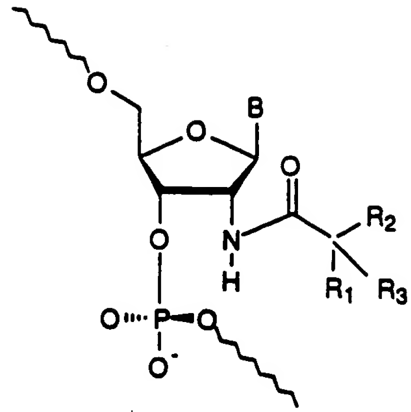
The triphosphate derivatives of the above nucleotides can be formed as shown in Fig. 89, according to known procedures. *Nucleic Acid Chem.*, Leroy B. Townsend, John Wiley & Sons, New York 1991, pp. 337-340; *Nucleotide Analogs*, Karl Heinz Scheit; John Wiley & Sons New York 1980, pp. 211-218.

Equivalent synthetic schemes for 3' dihalophosphonates are shown in Figures 90 and 91 using art recognized nomenclature. The conditions can be optimized by standard procedures.

The nucleoside dihalophosphonates described herein are advantageous as modified nucleotides in any nucleic acid structure, e.g., catalytic or antisense, since they are resistant to exo- and endonucleases that normally degrade unmodified nucleic acids *in vivo*. They also do not perturb the normal structure of the nucleic acid in which they are incorporated thereby maintaining any activity associated with that structure. These compounds may also be of use as monomers as antiviral and/or antitumor drugs.

Oligonucleotides with Amido or Peptido Modification

This invention replaces 2'-hydroxyl group of a ribonucleotide moiety with a 2'-amido or 2'-peptido moiety. In other embodiments, the 3' and 5' portions of the sugar of a nucleotide may be substituted, or the phosphate group may be substituted with amido or peptido moieties. Generally, such a nucleotide has the general structure shown in Formula I below:



FORMULA I

The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In addition, either R_1 or R_2 is H or an alkyl, alkene or alkyne group containing between 2 and 10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, e.g., R_3NR_4 where each R_3 and R_4 independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, i.e., an amide), an alkyl group, or an amino acid (D or L forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R_1, R_2 and R_3 is an H, and the other is an amino acid or peptide.

Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and interfere with standard base pairing interactions. Such interference will allow the formation of a compl x nucleic acid and protein conglomerate.

Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly generated oligonucleotides which can be folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in one aspect, the invention features an oligonucleotide containing the modified base shown in Formula I, above.

In other aspects, the oligonucleotide may include a 3' or 5' nucleotide having a 3' or 5' located amino acid or aminoacyl group. In all these aspects, as well as the 2'-modified nucleotide, it will be evident that various standard modifications can be made. For example, an "O" may be replaced with an S, the sugar may lack a base (i.e., abasic) and the phosphate moiety may be modified to include other substitutions (see Sproat, supra).

Example 93: General procedure for the preparation of 2'-aminoacyl-2'-deoxy-2'-aminonucleoside conjugates.

Referring to Fig. 92, to the solution of 2'-deoxy-2'-amino nucleoside (1 mmol) and N-Fmoc L- (or D-) amino acid (1 mmol) in methanol [dimethylformamide (DMF) and tetrahydrofuran (THF) can also be used], 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [or 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)] (2 mmol) is added and the reaction mixture is stirred at room temperature or up to 50 °C from 3-48 hours. Solvents are removed under reduced pressure and the residual syrup is chromatographed on the column of silica-gel using 1-10 % methanol in dichloromethane. Fractions containing the product are concentrated yielding a white foam with yields ranging from 85 to 95 %. Structures are confirmed by ^{1}H NMR spectra of conjugates which show correct chemical shifts for nucleoside and aminoacyl part of the molecule. Further proofs of the structures are obtained by cleaving the aminoacyl protecting groups under appropriate conditions and assigning ^{1}H NMR resonances for the fully deprotected conjugate.

Partially protected conjugates described above are converted into their 5'-O-dimethoxytrityl derivatives and into 3'-phosphoramidites using standard procedures (Oligonucleotide Synthesis: A Practical Approach,

M.J. Gait ed.; IRL Press, Oxford, 1984). Incorporation of these phosphoramidites into RNA was performed using standard protocols (Usman *et al.*, 1987 *supra*).

A general deprotection protocol for oligonucleotides of the present invention is described in Fig. 93.

The scheme shows synthesis of conjugate of 2'-d-2'-aminouridine. This is meant to be a non-limiting example, and those skilled in the art will recognize that, variations to the synthesis protocol can be readily generated to synthesize other nucelotides (e.g., adenosine, cytidine, guanosine) and/or abasic moieties.

Example 94: RNA cleavage by hammerhead ribozymes containing 2'-aminoacyl modifications.

Hammerhead ribozymes targeted to site N (see Fig. 94) are synthesized using solid-phase synthesis, as described above. U4 and U7 positions are modified, individually or in combination, with either 2'-NH-alanine or 2'-NH-lysine.

RNA cleavage assay *in vitro*: Substrate RNA is 5' end-labeled using [γ-^{32}P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount ($\leq$ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM $MgCl_2$. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μl are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Fig. 95, hammerhead ribozymes containing 2'-NH-alanine or 2'-NH-lysine modifications at U4 and U7 positions cleave the target RNA efficiently.

Sequences listed in Figure 94 and the modifications described in Figure 95 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 95: Aminoacylation of 3'-ends of RNA

I. Referring to Fig. 96, 3'-OH group of the nucleotide is converted to succinate as described by Gait, *supra*. This can be linked with amino-alkyl solid support (for example: CpG). Zig-zag line indicates linkage of 3'OH group with the solid support.

II. Preparation of aminoacyl-derivatized solid support

A) Synthesis of O-Dimethoxytrityl (O-DMT) amino acids

Referring to Fig. 97, to a solution of L- (or D-) serine, tyrosine or threonine (2 mmol) in dry pyridine (15 ml) 4,4'-dimethoxytrityl chloride (3 mmol) is added and the reaction mixture is stirred at RT (about 20-25°C) for 16 h. Methanol (10 ml) is then added and the solution evaporated under reduced pressure. The residual syrup was partitioned between 5% aq. $NaHCO_3$ and dichloromethane, organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue is purified by flash silicagel column chromatography using 2-10% methanol in dichloromethane (containing 0.5 % pyridine). Fractions containing product are combined and concentrated *in vacuo* to yield white foam (75-85 % yield).

B) Preparation of the solid support and its derivatization with amino acids

Referring to Fig. 97, the modified solid support (has an OH group instead of the standard NH_2 end group) was prepared according to Haralambidis et al., Tetrahedron Lett. 1987, 28, 5199, (P denotes aminopropyl CPG or polystyrene type support). O-DMT or NH-monomethoxytrityl (NH-MMT amino acid was attached to the above solid support using standard procedures for derivatization of the solid support (Gait, 1984, supra) creating a bas -labile ester bond between amino acids

and th support. This support is suitable for the construction of RNA/DNA chain using suitably protected nucleoside phosphoramidites.

Example 96: Aminoacylation of 5'-ends of RNA

I. Referring to Fig. 98, 5'-amino-containing sugar moiety was synthesized as described (Mag and Engels, 1989 *Nucleic Acids Res.* 17, 5973). Aminoacylation of the 5'-end of the monomer was achieved as described above and RNA phosphoramidite of the 5'-aminoacylated monomer was prepared as described by Usman *et al.*, 1987 *supra*. The phosphoramidite was then incorporated at the 5'-end of the oligonucleotide using standard solid-phase synthesis protocols described above.

II. Referring to Fig. 99, aminoacyl group(s) is attached to the phosphate group at the 5'-end of the RNA using standard procedures described above.

VII. Reversing Genetic Mutations

Modification of existing nucleic acid sequences can be achieved by homologous recombination. In this process a transfected sequence recombines with homologous chromosomal sequences and can replace the endogenous cellular sequence. Boggs, 8 *International J. Cell Cloning* 80, 1990, describes targeted gene modification. It reviews the use of homologous DNA recombination to correct genetic defects. Banga and Boyd, 89 *Proc. Natl. Acad. Sci. U.S.A.* 1735, 1992, describe a specific example of *in vivo* site-directed mutagenesis using a 50 base oligonucleotide. In this methodology a gene or gene segment is essentially replaced by the oligonucleotide used.

This invention uses a complementary oligonucleotide to position a nucleotide base changing activity at a particular site on a gene (RNA or genomic DNA), such that the nucleotide modifying activity will change (or revert) a mutation to wild-type, or its equivalent. By reversion or change of a mutation, we refer to reversion in a broad sense, such as when a mutation at a second site which leads to functional reversion to a wild type phenotype. Also, due to the degeneracy of the genetic code, a revertant may be achieved by changing any one of the three codon positions. Additionally, creation of a stop codon in a deleterious gene (or transcript) is defined here as r verting a mutant phenotype to wild-type. An example of

this type of reversion is creating a stop codon in a critical HIV proviral gene in a human.

Referring to Figures 100 and 101, broadly there are two approaches to causing a site directed change in order to revert a mutation to wild-typ . In one (Fig. 100) the oligonucleotide is used to target RNA specifically. RNA is provided with a complementary (Watson-crick) oligonucleotide sequence to that in the target molecule. In this case the sequence modifying oligonucleotide would (analogously to an antisense oligonucleotide or ribozyme) have to be continuously present to revert the RNA as it is made by the cell. Such a reversion would be transient and would potentially require continuous addition of more sequence modifying oligonucleotide. The transient nature of this approach is an advantage, in that treatment could be stopped by simply removing the sequence modifying oligonucleotide (as with a traditional drug).

A second approach targets DNA (Fig. 101) and has the advantage that changes may be permanently encoded in the target cell's genetic code. Thus, a single course (or several courses) of treatment may lead to permanent reversion of the genetic disease. If inadvertent chromosomal mutations are introduced this may cause cancer, mutate other genes, or cause genetic changes in the germ-line (in patients of reproductive age). However, if the base changing activity is a specific methylation that may modulate gene expression it would not necessarily lead to germ-line transmission. See Lewin, Genes, 1983 John Wilely & Sons, Inc. NY pp 493-496.

Complementary base pairing to single-stranded DNA or RNA is one method of directing an oligonucleotide to a particular site of DNA. This could occur by a strand displacement mechanism or by targeting DNA when it is single-stranded (such as during replication, or transcription). Another method is using triple-strand binding (triplex formation) to double-stranded DNA, which is an established technique for binding poly-pyrimidine tracts, and can be extended to recognize all 4 nucleotides. See Povsic, T., Strobel, S., & Dervan, P. (1992). Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation. J. Am. Chem. Soc. 114, 5934-5944 (1992). Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivativ s* 1-366 (CRC Press, Novosibirsk,

1993) describe conjugation of reactive groups or enzyme to oligonucleotides and can be used in the methods described herein.

Recently, antisense oligonucleotides have been used to redirect an incorrect splice into order to obtain correct splicing of a splice mutant globin gene *in vitro.* Dominski Z; Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U S A 90:8673-7. Analogously, in one preferred embodiment of this invention a complementary oligomer is used to correct an existiing mutant RNA, instead of the traditional approach of inhibiting that RNA by antisense.

In either the RNA or DNA mode, after binding to a particular site on the RNA or DNA the oligonucleotide will modify the nucleic acid sequence. This can be accomplished by activating an endogenous enzyme (see Figure 102), by appropriate positioning of an enzyme (or ribozyme) conjugated (or activated by the duplex) to the oligonucleotide, or by appropriate positioning of a chemical mutagen. Specific mutagens, such as nitrous acid which deaminates C to U, are most useful, but others can also be used if inactivation of a harmful RNA is desired.

RNA editing is an naturally occurring event in mammalian cells in which a sequence modifying activity edits a RNA to its proper sequence post-transcriptionally. Higuchi, M.,, Single, F., Kohler, M., Sommer, B., and Seeburg, P. (1993) RNA Editing of AMPA Receptor Subunit GluR-B: A base-paired intron-exon structure determines position and efficiency Cell 75:1361-1370. The machinery involved in RNA editing can be co-opted by a suitable oligonucleotide in order to promote chemical modification.

The changes in the base created by the methods of this invention cause a change in the nucleotide sequence, either directly, or after DNA repair by normal cellular mechanisms. These changes functionally correct a genetic defect or introduce a stop codon. Thus, the invention is distinct from techniques in which an active chemical group (*e.g.*, an alkylator) is attached to an antisense or triple strand oligonucleotide in order to chemically inactivate the target RNA or DNA.

Thus, this invention creates an alteration to an existing base in a nucleic acid molecule so that the base is r ad *in vivo* as a different base.

This includes correcting a sequence instead of inactivating a gene but can also include inactivating a deleterious gene.

Thus, in one aspect, the invention features a method for altering <u>in vivo</u> the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule. The method includes contacting the nucleic acid molecule <u>in vivo</u> with an oligonucleotide or peptide nucleic acid or other sequence specific binding molecules able to form a duplex or triplex molecule with the nucleic acid molecule. After formation of the duplex or triplex molecule a base modifying activity chemically or enzymatically alters the targeted base directly, or after nucleic acid repair *in vivo*. This results in the functional alteration of the nucleic acid sequence.

By "alter", as it is used in this context, is meant that one or more chemical moieties in a targeted base, or bases, is altered so that the mutant nucleic acid will be functionally different. Thus, this is distinct from prior methods of correcting defects in DNA, such as homologous recombination, in which an entire segment of the targeted sequence is replaced with a segment of DNA from the transfected nucleic acid. This is also distinct from other methods that use reactive groups to inactivate a RNA or DNA target, in that this method functionally corrects the sequence of the target, instead of merely damaging it, by causing it to be read by a polymerase as a different base from the original base. As noted above, the naturally occurring enzymes in a cell can be utilized to cause the chemical alteration, examples of which are provided below.

By "functionally alter" is meant that the ability of the target nucleic acid to perform its normal function (*i.e.*., transcription or translation control) is changed. For example, an RNA molecule may be altered so that it can cause production of a desired protein, or a DNA molecule can be altered so that upon DNA repair, the DNA sequence is changed.

By "mutant" it is meant a nucleic acid molecule which is altered in some way compared to equivalent molecules present in a normal individual. Such mutants may be well known in the art, and include, molecules present in individuals with known genetic deficiencies, such as muscular dystrophy, or diabetes and the like. It also includes individuals with diseases or conditions characterized by abnormal expression of a gene, such as cancer, thalassemia's and sickle cell anemia, and cystic

fibrosis. It allows modulation of lipid metabolism to reduce artery disease, treatment of integrated AIDS genomes, and AIDs RNA, and Alzeimer's disease. Thus, this invention concerns alteration of a base in a mutant to provide a "wild type" phenotype and/or genotype. For deleterious conditions this involves altering a base to allow expression or prevent expression as is necassary. When treating an infection, such as HIV, it concerns inactivation of a gene in the HIV RNA by mutation of the mutant (*i.e.*, non-human gene) to a wild type (*i.e.*, no production of a non-human protein). Such modification is performed *in trans* rather than *in cis* as in prior methods.

In preferred embodiments, the oligonucleotide is of a length (at least 12 bases, preferably 17 - 22) sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine; the oligonucleotide is an enzymatic nucleic acid molecule that is active to chemically modify a base (see below); the nucleic acid molecule is DNA or RNA; the oligonucleotide includes a chemical mutagen, *e.g.*, the mutagen is nitrous acid; and the oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methtylation of cytosine to 5-methylcytosine.

In a most preferred embodiment, the invention features correction of a mutation, rather than inactivation of a target by causing a mutation.

Using *in vitro* directed evolution, it is possible to screen for ribozymes with catalytic activities different than RNA cleavage. Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418. Using these methods of *in vitro* directed evolution, an enzymatic nucleic acid molecule, or ribozyme that mutates bases, instead of cleaving the phosphodiester backbone can be selected. This is a convenient method of obtaining an enzyme with the appropriate base sequence modifying activities for use in the present invention.

Sequence modifying activities can change one nucleotide to another (or modify a nucleotide so that it will be repaired by the cellular machinery to another nucleotide). Sequence modifying activities could also delete or add one or more nucleotides to a sequence. A specific embodiment of adding sequences is described by Sullenger and Cech, PCT/US94/12976

hereby incorporated by reference herein), in which entire exons with wild-type sequence are spliced into a mutant transcript. The present invention features only the addition of a few bases (1 - 3).

Thus, in another aspect, the invention features ribozymes or enzymatic nucleic acid molecules active to change the chemical structure of an existing base in a separate nucleic acid molecule. Applicant is the first to determine that such molecules would be useful, and to provide a description of how such molecules might be isolated.

Molecules used to achieve *in situ* reversion can be delivered using the existing means employed for delivering antisense molecules and ribozymes, including liposomes and cationic lipid complexes. If the *in situ* reverting molecule is composed only of RNA, then expression vectors can be used in a gene therapy protocol to produce the reverting molecules endogenously, analogously to antisense or ribozymes expression vectors. There are several advantages of using such an expression vector, rather than simply replacing the gene through standard gene therapy. Firstly, this approach would limit the production of the corrected gene to cells that already express that gene. Furthermore, the corrected gene would be properly regulated by its natural transcriptional promoter. Lastly, reversion can be used when the mutant RNA creates a dominant gain of function protein (*e.g.*, in sickle cell anemia), where correction of the mutant RNA is necessary to stop the production of the deleterious mutant protein, and allow production of the corrected protein.

Endogenous Mammalian RNA Editing System

It was observed in the mid-1980s that the sequence of certain cellular RNAs were different from the DNA sequence that encodes them. By a process called RNA editing, cellular RNA are post-transcriptionally modified to a) create a translation initiation and termination codons, b) enable tRNA and rRNA to fold into a functional conformation (for a review see Bass, B. L. (1993) In The RNA World, R. Gesteland, R. and Atkins, J. eds. (Cold Spring Harbor, New York; CSH Lab. Press) pp. 383-418). The process of RNA editing includes base modification, deletion and insertion of nucleotides.

Although, the RNA editing process is widespread among lower eukaryotes, very few RNAs (four) have been reported to undergo editing in

mammals (Bass, *supra*). The predominant mode of RNA editing in mammalian system is base modification (C → U and A → G). The mechanism of RNA editing in the mammalian system is postulated to be that C→U conversion is catalyzed by cytidine deaminase. The mechanism of conversion of A→G has recently been reported for glutamate receptor B subunit (gluR-B) in rat PC12 cells (Higuchi, M. et al. (1993) Cell 75, 1361-1370). According to Higuchi gluR-B mRNA precursor attains a structure such that intron 11 and exon 11 can form a stable stem-loop structure. This stem-loop structure is a substrate for a nuclear double strand-specific adenosine deaminase enzyme. The deamination will result in the conversion of A→I. Reverse transcription followed by double strand synthesis will result in the incorporation of G in place of A.

In the present invention, the endogenous deaminase activity or other such activities can be utilized to achieve targeted base modification.

The following are examples of the invention to illustrate different methods by which *in vivo* conversion of a base can be achieved. These are provided only to clarify specific embodiments of the invention and are not limiting to the invention. Those in the art will recognize that equivalent methods can be readily devised within the scope of the claims.

Example 97: Exploiting cellular dsRNA dependent Adenine to Inosine converter:

An endogenous activity in most mammalian cells and Xenopus oocytes converts about 50% of adenines to inosines in double stranded RNA. (Bass, B. L., & Weintraub, H. (1988). An unwinding activity that covalently modifies it double-stranded RNA substrate. Cell, 55, 1089-1098.). This activity can be used to cause an *in situ* reversion of a mutation at the RNA level. Referring to Figures 102 and 104, for demonstration purposes a stop codon is incorporated into the coding region of dystrophin, which is fused to the reporter gene luciferase. This stop codon can be reverted by targeting an antisense RNA which is long enough to activate the dsRNA deaminase, which converts Adenines to Inosines. The A to I transition will be read by the ribosome as an A to G transition in some cases and will thereby functionally revert the stop codon. While other A's in this region may be converted to I's and read as G, converting an A to I (G) cannot create a stop codon. The A to I transitions

in the region surrounding the target mutation will create some point mutations, however, the function of the dystrophin protein is rarely inactivated by point mutations.

The reverted mRNA was then translated in a cell lysate and assayed for luciferase activity. As evidenced by the dramatic increase in luciferase counts in the graph in figure 103, the A to I transition was read by the ribosome as an A to G transition and the stop codon has successfully been reverted with the lysate treated complex. As a control, an irrelevant non-complementary RNA oligonucleotide was added to the dystrophin/luciferase mRNA. As expected, in this case no translation (luciferase activity) is observed because of the stop codon. As an additional control, the hybrid was not treated with extract, and again no translation (luciferase activity) is observed (Figure 103).

While other A's in the targeted region may have been converted to I's and read as G, converting an A to I (G) cannot create a stop codon, so the ribosome will still read through the region. Dystrophin is not generally sensitive to point mutations if the open reading frame is maintained, so a dystrophin protein made from an mRNA reverted by this method should retain full activity.

The following detail specifics of the methodology: RNA oligonucleotides were synthesized on a 394 (ABI) synthesizer using phosphoramidite chemistry. The sequence of the synthetic complementary RNA that binds to the mutant dystrophin sequence is as follows (5' to 3'):

CCCGCGGTAGATCTTTCTGGAGGCTTACAGTTTTCTACAAACCTCC CTTCAAA (Seq. ID No. 1)

Referring to <u>Figure 104</u>, fifty-nine base pairs of a human dystrophin mutant sequence containing a stop codon was fused in frame to the luciferase coding region using standard cloning technology, into the *Hin*d III and *Not* I sites of pRC-CMV (Invitrogen, San Diego, CA). The AUG of luciferase was deleted. The sequences of the insert from the *Hin*d III site to the start of the luciferase coding region is (5' to 3'):

GCCCCTGAGGAGCGATGGAGGCCTTGAAGGGAGGTTTGTGGAAAA

CTGTAAGCCTCCAGAAAGATCTACCGCGG (Seq ID No. 2)

This corresponds to base pairs 3649-3708 of normal dystrophin (Entrez ID # 311627) with a *Sac* II site at the 3' end. This plasmid was used as a template for *in vitro* transcription of mRNA using T7 polymerase with the manufacturers protocol (Promega, Madison, WI).

Xenopus nuclear extracts were prepared in 0.5X TGKED buffer (0.5X= 25mM Tris (pH 7.9), 12.5% glycerol, 25 mM KCl, 0.25mM DTT and 0.05mM EDTA), by vortexing nuclei and resuspended in a volume of 0.5X TGKED equal to total cytoplasm volume of the oocytes. Bass, B.L. & Weintraub, H. *Cell* 55, 1089-1098 (1988).

The target mRNA at 500ng/ul was pre-annealed to 1 micromolar complementary or irrelevant RNA oligonucleotide by heating to 70°C, and allowing it to slowly cool to 37°C over 30 minutes. Fifty nanograms of mRNA pre-annealed to the RNA oligonucleotides was added to 7ul of nuclear extracts containing 1mM ATP, 15mM EDTA, 1600un/ml RNasin and 12.5mM Tris pH 8 to a total volume of 12ul. Bass, B.L. & Weintraub, H. *supra*. This mixture, which contains the dsRNA deaminase activity, was incubated for 30 minutes at 25°C. Next, 1.5ul of this mixture was added to a rabbit reticulocyte lysate *in vitro* translation mixture and translated for two hours according to the manufacturers protocol (Life Technologies, Gaithersberg, MD), except that an additional 1.3 mM magnesium acetate was added to compensate for the EDTA carried through from the nuclear extract mixture. Luciferase assays were performed on 15ul of extract with the Promega luciferase assay system (Promega, Madison, WI), and luminescence was detected with a 96 well luminometer, and the results are displayed in the graph in figure 102.

Example 98: Base changing activities

The chemical synthesis of antisense and triple-strand forming oligomers conjugated to reactive groups is well studied and characterized (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993) and Povsic, T., Strobel, S. & Dervan, P. Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation *J. Am. Chem. Soc.* 114, 5934-5944 (1992). Reactive groups such as alkylators that can modify nucleotide bases in targeted RNA or DNA have been conjugated to oligonucleotides.

Additionally enzymes that modify nucleic acids have be n conjugated to oligonucleotides. (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993). In the past these conjugated chemical groups or enzymes have been used to inactivate DNA or RNA that is specifically targeted by antisense or triple-strand interactions. Below is a list of useful base changing activities that could be used to change the sequence of DNA or RNA targeted by antisense or triple strand interactions, in order to achieve *in situ* reversion of mutations, as described herein (see figure 100-104).

1. Deamination of 5-methylcytosine to create thymidine (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993). Also, nitrous acid or related compounds promote oxidative deamination of C to be read at T(Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston,1987, PP.226-230.). Additionally hydroxylamine or related compounds can transform C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston,1987, PP.226-230.)

2. Deamination of cytosine to create uracil (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993) or by chemical groups similar to nitrous acid that promote oxidative deamination (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston,1987, PP.226-230.)

3. Deamination of Adenine to be read like G (Inosine) (as done by the adenosine deaminase, AMP deaminase or the dsRNA deaminating activity (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

4. Methylation of cytosine to 5-methylcytosine

5. Transforming thymidine (or uracil) to O^2-methyl thymidine (or O^2-methyl uracil), to be read as cytosine by alkynitrosoureas (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).

6. Transforming guanine to 6-*O*-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978) which can be done with the mutagen ethyl methane sulfonate (EMS) Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.

7. Amination of uracil to cytosine (as performed by the cellular enzyme CTP synthetase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

The following are examples of useful chemical modifications that can be utilized in the present invention. There are a few preferred straightforward chemical modifications that can change one base to another base. Appropriate mutagenic chemicals are placed on the targetting oligonucleotide, *e.g.*, nitrous acid, or a suitable protein with such activity. Such chemicals and proteins can be attatched by standard procedures. These include molecules which introduce fundamental chemical changes, that would be useful independent of the particular technical approach. See Lewin, Genes, 1983 John Wilely & Sons, Inc. NY pp 42-48.

The following matrix shows that the chemical modifications noted can cause transversion reversions (pyrimidine to pyrimidine, or purine to purine) in RNA or DNA. The transversions (pyrimidine to purine, or purine to pyrimidine) are not preferred because these are more difficult chemical transformations. The footnotes refer to the specific desired chemical transformations. The bold footnotes refer to the reaction on the opposite DNA strand. For example, if one desires to change an A to a G, this can be accomplished at the DNA level by using reaction #5 to change a T to a C in the opposing strand. In this example an A/T base pair goes to A/C, then when the DNA is replicated, or mismatch repair occurs this can become G/C, thus the original A has been converted to a G.

ISR matrix

Reverted Base

Mutant base	A	T(U)	C	G

A	-	Transversion	Transversion	DNA[5,3]/RNA[3]
T(U)	Transversion	-	DNA[5]/RNA[7]	Transversion
C	Transversion	RNA[2]/DNA[6]	-	Transversion
G	DNA[6]/RNA[6]	Transversion	Transversion	-

1 Deamination of 5-methylcytosine to create thymidine.

2 Deamination of cytosine to create uracil.

3 Deamination of Adenine to be read like G (Inosine).

4 Methylation of cytosine to 5-methylcytosine.

5 Transforming thymidine (or uracil) to O^2-methyl thymidine (or O^2-methyl uracil), to be read as cytosine (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).

6 Transforming guanine to 6-*O*-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978)).

7. Amination of uracil to cytosine. Bass *supra.* fig. 6c.

In Vitro Selection Strategy

Referring to Figure 105, there is provided a schematic describing an approach to selecting for a ribozyme with such base changing activity. An RNA is designed that folds back on itself (this is similar to approaches already used to select for RNA ligases, Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418). A degenerate loop opposing the base to be modified provides for diversity. After incubating this library of molecules in a buffer, the RNA is reverse transcribed into DNA (that is, using standard *in vitro* evolution protocol. Tuerk and Gold, 249 Science 505, 1990) , and then the DNA is selected for having a base change. A restriction enzyme cleavage and size selection or its equivalent is used to isolate the fraction of DNAs with the appropriate base change. The cycle could then be repeated many tim s.

The *in vitro* selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641; Joyce, G. F. (1992) Scientific American 267, 90-97) and Szostak (Bartel, D. and Szostak, J. (1993) Science 261:1411-1418; Szostak, J. W. (1993) TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (the region flanking the mutant nucleotide), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their base modifying activity, 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences (see Figure 105), such that the degenerate domain is placed across from the mutant base (the base that is targeted for modification). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the catalysis of base modification (the reaction protocol may also include certain cofactors like ATP or GTP or an S-adenosyl-methionine (if methylation is desired) in order to make the selection more stringent). Following incubation, nucleic acids are converted into complimentary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with base modification (at the mutant base position) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a restriction endonuclease cleavage site can either be created or abolished as a result of base modification. If a restriction endonuclease site is created as a result of base modification, then the library can be digested with the restriction endonuclease (RE). The fraction of the population that is cleaved by the RE is the population that has been able to catalyze the base modification reaction (active pool). A new piece of DNA (containing oligonucleotide primer binding sites for PCR and RE sites for cloning) is ligated to the termini of the active pool to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the best base modifying activity is cloned in to a plasmid vector

and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Base modifying enzymatic nucleic acids (identified via in vitro selection) can be used to cause the chemical modification *in vivo.*

In addition, the ribozyme could be evolved to specifically bind a protein having an enzymatic base changing acitivity.

Such ribozymes can be used to cause the above chemical modifications *in vivo.* The ribozymes or above noted antisense-type molecules can be administered by methods discussed in the above referenced art.

VIII. Administration of Nucleic Acids

Applicant has determined that double-stranded nucleic acid lacking a transcription termination signal can be used for continuous expression of the encoded RNA. This is achieved by use of an R-loop, *i.e.*, an RNA molecule non-covalently associated with the double-stranded nucleic acid and which causes localized denaturation ("bubble" formation) within the double stranded nucleic acid (Thomas et al., 1976 Proc. Natl. Acad. Sci. USA 73, 2294). In addition, applicant has determined that that the RNA portion of the R-loop can be used to target the whole R-loop complex to a desirable intracellular or cellular site, and aid in cellular uptake of the complex. Further, applicant indicates that expression of enzymatically active RNA or ribozymes can be significantly enhanced by use of such R-loop complexes.

Thus, in one aspect, the invention features a method for introduction of enzymatic nucleic acid into a cell or tissue. A complex of a first nucleic acid encoding the enzymatic nucleic acid and a second nucleic acid molecule is provided. The second nucleic acid molecule has sufficient complementarity with the first nucleic acid to be able to form an R-loop base pair structure under physiological conditions. The R-loop is formed in a region of the first nucleic acid molecule which promotes expression of RNA from the first nucleic acid under physiological conditions. The method further includes contacting the complex with a cell or tissue under

conditions in which the enzymatic nucleic acid is produced within the cell or tissue.

By "complex" is simply meant that the two nucleic acid molecules interact by intermolecular bond formation (such as by hydrogen bonding) between two complementary base-paired sequences. The complex will generally be stable under physiological condition such that it is able to cause initiation of transcription from the first nucleic acid molecule.

The first and second nucleic acid molecules may be formed from any desired nucleotide bases, either those naturally occurring (such as adenine, guanine, thymine and cytosine), or other bases well known in the art, or may have modifications at the sugar or phosphate moieties to allow greater stability or greater complex formation to be achieved. In addition, such molecules may contain non-nucleotides in place of nucleotides. Such modifications are well known in the art, see *e.g.,* Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science, 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Sproat,B. *European Patent Application 92110298.4* which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.

By "sufficient complementarity" is meant that sufficient base pairing occurs so that the R-loop base pair structure can be formed under the appropriate conditions to cause transcription of the enzymatic nucleic acid. Those in the art will recognize routine tests by which such sufficient base pairs can be determined. In general, between about 15 - 80 bases is sufficient in this invention.

By "physiological condition" is meant the condition in the cell or tissue to be targeted by the first nucleic acid molecule, although the R-loop complex may be formed under many other conditions. One example is use of a standard physiological saline at 37°C, but it is simply desirable in this invention that the R-loop structure exists to some extent at the site of action so that the expression of the desired nucleic acid will be achieved at that site of action. While it is preferred that the R-loop structure be stable under

those conditions, even a minimal amount of formation of the R-loop structure to cause expression will be sufficient. Those in the art will recognize that measurement of such expression is readily achieved, especially in the absence of any promoter or leader sequence on the first nucleic acid molecule (Daube and von Hippel, 1992 Science 258, 1320). Such expression can thus only be achieved if an R-loop structure is truly formed with the second nucleic acid. If a promoter of leader sequence is provided, then it is preferred that the R-loop be formed at a site distant from those regions so that transcription is enhanced.

In a related aspect, the invention features a method for introduction of ribonucleic acid within a cell or tissue by forming an R-loop base-paired structure (as described above) with the first nucleic acid molecule lacking any promoter region or transcription termination signal such that once expression is initiated it will continue until the first nucleic acid is degraded.

In another related aspect, the invention features a method in which the second nucleic acid is provided with a localization factor, such as a protein, *e.g.*, an antibody, transferin, a nuclear localization peptide, or folate, or other such compounds well known in the art, which will aid in targeting the R-loop complex to a desired cell or tissue.

In preferred embodiments, the first nucleic acid is a plasmid, *e.g.*, one without a promoter or a transcription termination signal ; the second nucleic acid is of length between about 40-200 bases and is formed of ribonucleotides at a majority of positions; and the second nucleic is covalently bonded with a ligand such as a nucleic acid, protein, peptide, lipid, carbohydrate, cellular receptor, nuclear localization factor, or is attached to maleimide or a thiol group: the first nucleic acid is an expression plasmid lacking a promoter able to express a desired gene, *e.g.*, it is a double-stranded molecule formed with a majority of deoxyribonucleic acids; the R-loop complex is a RNA/DNA heteroduplex; no promoter or leader region is provided in the first nucleic acid; and the R-loop is adapted to prevent nucleosome assembly and is designed to aid recruitment of cellular transcription machinery.

In other preferred embodiments, the first nucleic acid encodes one or more enzymatic nucleic acids, *e.g.*, it is formed with a plurality of

intramolecular and intermolecular cleaving enzymatic nucleic acids to allow release of therapeutic enzymatic nucleic acid *in vivo*.

In a further related aspect, the invention features a complex of the above first nucleic acid molecules and second nucleic acid molecules.

R-loop complex

An R-loop complex is designed to provide a non-integrating plasmid so that, when an RNA polymerase binds to the plasmid, transcription is continuous until the plasmid is degraded. This is achieved by hybridizing an RNA molecule, 40 to 200 nucleotides in length, to a DNA expression plasmid resulting in an R-loop structure (see figure 106). This RNA, when conjugated with a ligand that binds to a cell surface receptor, triggers internalization of the plasmid/RNA-ligand complex. Formation of R-loops in general is described by DeWet, 1987 Methods in Enzymol. 145, 235; Neuwald et al., 1977 J. Virol. 21,1019; and Meyer et al., 1986 J. Ult. Mol. Str. Res. 96, 187. Thus, those in the art can readily design complexes of this invention following the teachings of the art.

Promoters placed in retroviral genomes have not always behaved as planned in that the additional promoter will serve as a stop signal or reverses the direction of the polymerase. Applicant was told that creation of an R-loop between the promoter and the reporter gene increased the transfection efficiency. Incubation of an RNA molecule with a double-stranded DNA molecule, containing a region of complementarity with the RNA will result in the formation of a stable RNA-DNA hetroduplex and the DNA strand that has a sequence identical to the RNA will be displaced into a loop-like structure called the R-loop. This displacement of DNA strand occurs because an RNA-DNA duplex is more stable compared to a DNA-DNA duplex. Applicant was also told that an 80 nt long RNA was used to generate a R-loop structure in a plasmid encoding the ß-galactosidase gene. The R-loop was initiated either in the promoter region or in the leader sequence. Plasmids containing an R-loop structure were microinjected into the cytoplasm of COS cells and the gene expression was assayed. R-loop formation in the promoter region of the plasmid inhibited expression of the gene. RNA that hybridized to the leader sequence between the promoter and the gene, or directly to the first 80 nucleotides of the mRNA increased the expression levels 8-10 fold. The

proposed mechanism is that R-loop formation prevents nucleosome assembly, thus making the DNA more accessible for transcription. Alternatively, the R-loop may resemble a RNA primer promoting either DNA replication or transcription (Daube and von Hippel, 1992, supra).

One of the salient features of this invention is to generate R-loops in expression vectors of choice and introduce them into cells to achieve enhanced expression from the expression vector. The presence of an R-loop may aid in the recruitment of cellular transcription machinery. Once an RNA polymerase binds to the plasmid and initiates transcription, the process will continue until a termination signal is reached, or the plasmid is degraded.

This invention will increase the expression of ribozymes inside a cell. The idea is to construct a plasmid with no transcription termination signal, such that a transcript-containing multiple ribozyme units can be generated. In order to liberate unit length ribozymes, self-processing ribozymes can be cloned downstream of each therapeutic ribozyme (see figure 107) as described by Draper *supra*.

Ligand Targeting

Another salient feature of this invention is that the RNA used to generate R-loop structures can be covalently linked to a ligand (nucleic acid, proteins, peptides, lipids, carbohydrates, *etc.*). Specific ligands can be chosen such that the ligand can bind selectively to a desired cell surface receptor. This ligand-receptor interaction will help internalize a plasmid containing an R-loop. Thus, RNA is used to attach the ligand to the DNA such that localization of the gene to certain regions of the cell is achieved. One of several methods can be used to attach a ligand to RNA. This includes the incorporation of deoxythymidine containing a 6 carbon spacer having a terminal primary amine into the RNA (see figure 108). This amino group can be directly derivatized with the ligand, such as folate (Lee and Low, 1994 J. Biol. Chem. 269, 3198-3204). The RNA containing a 6 carbon spacer with a terminal amine group is mixed with folate and the mixture is reacted with activators like 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). This reaction should be carried out in the presence of 1-Hydroxybenzotriazole hydrate (HOBT) to prevent any undesirable side reactions.

The RNA can also be derivatized with a heterobifuctional crosslinking agent (or linker) like succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB). The SMPB introduces a maleimide into the RNA. This maleimide can then react with a thiol moiety either in a peptide or in a protein. Thiols can also be introduced into proteins or peptides that lack naturally occurring thiols using succinylacetylthioacetate. The amino linker can be attached at the 5' end or 3' end of the RNA. The RNA can also contain a series of nucleotides that do not hybridize to the DNA and extend the linker away from the RNA/DNA complex, thus increasing the accessibility of the ligand for its receptor and not interfering with the hybridization. These techniques can be used to link peptides such as nuclear localization signal (NLS) peptides (Lanford et al., 1984 Cell 37, 801-813; Kalderon et al., 1984 Cell 39, 499-509; Goldfarb et al., 1986 Nature 322, 641-644)and/or proteins like the transferrin (Curiel et al., 1991 Proc. Natl. Acad. Sci. USA 88, 8850-8854; Wagner et al., 1992 Proc. Natl. Acad. Sci. USA 89, 6099-6103; Giulio et al., 1994 Cell. Signal. 6, 83-90) to the ends of R-loop forming RNA in order to facilitate the uptake and localization of the R-loop-DNA complex. To link a protein to the ends of R-loop forming RNA, an intrinsic thiol can be used to react with the maleimide or the thiols can be introduced into the protein itself using either iminothiolate or succinimidyl acetyl thioacetate (SATA; Duncan et al., 1983 Anal. Biochem 132, 68). The SATA requires an additional deprotection step using 0.5 M hydroxylamine.

In addition liposomes can be used to cause an R-loop complex to be delivered to an appropriate intracellular cite by techniques well known in the art. For example, pH-sensitive liposomes (Connor and Huang, 1986 Cancer Res. 46, 3431-3435) can be used to facilitate DNA transfection.

Calcium phosphate mediated or electroporation-mediated delivery of the R-loop complex in to desired cells can also be readily acomplished.

In vitro Selection

In vitro selection strategies can be used to select nucleic acids that a) can form stable R-loops b) selectively bind to specific cell surface receptors. These nucleic acids can then be covalently linked to each other. This will help internalize the R-loop-containing plasmid efficiently using receptor-mediated endocytosis. The *in vitro* selection (evolution) strategy is

similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635-641; Joyce, 1992 Scientific American 267, 90-97) and Szostak (Bartel and Szostak, 1993 Science 261:1411-1418; Szostak, 1993 TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (a specific region of the double strand DNA), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their affinity to form R-loop and/or their ability to bind to a specific receptor, 3) introduction of a restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences. This random library of nucleic acids is incubated under conditions that ensure equilibrium binding to either double-stranded DNA or cell surface receptor. Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired characteristics can be separated from the rest of the population of nucleic acids by using a variety of methods (Joyce, 1992 supra). The desired pool of nucleic acids can then be carried through subsequent rounds of selection to enrich the population with the most desired traits. These molecules are then cloned in to appropriate vectors. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Other embodiments are within the following claims.

TABLE I

Characteristics of Ribozymes

Group I Introns
Size: ~200 to >1000 nucleotides.
Requires a U in the target sequence immediately 5' of the cleavage site.
Binds 4-6 nucleotides at 5' side of cleavage site.
Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)
Size: ~290 to 400 nucleotides.
RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.
Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme
Size: ~13 to 40 nucleotides.
Requires the target sequence UH immediately 5' of the cleavage site.
Binds a variable number nucleotides on both sides of the cleavage site.
14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figures 1 and 2)

Hairpin Ribozyme
Size: ~50 nucleotides.
Requires the target sequence GUC immediately 3' of the cleavage site.
Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.
Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, **arabis mosaic virus and chicory yellow mottle virus**) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme
Size: 50 - 60 nucleotides (at present).
Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.
Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table 2
Human ICAM HH Target sequence

nt. Position	Target Sequences	nt. Position	Target Sequences
11	CCCCAGU C GACGCUG	386	ACCGUGU A CUGGACU
23	CUGAGCU C CUCUGCU	394	CUGGACU C CAGAACG
26	AGCUCCU C UGCUACU	420	CACCCCU C CCCUCUU
31	CUCUGCU A CUCAGAG	425	CUCCCCU C UUGGCAG
34	UGCUACU C AGAGUUG	427	CCCCUCU U GGCAGCC
40	UCAGAGU U GCAACCU	450	AGAACCU U ACCCUAC
48	GCAACCU C AGCCUCG	451	GAACCUU A CCCUACG
54	UCAGCCU C GCUAUGG	456	UUACCCU A CGCUGCC
58	CCUCGCU A UGGCUCC	495	CCAACCU C ACCGUGG
64	UAUGGCU C CCAGCAG	510	UGCUGCU C CGUGGGG
96	CCGCACU C CUGGUCC	564	CUGAGGU C ACGACCA
102	UCCUGGU C CUGCUCG	592	GAGAGAU C ACCAUGG
108	UCCUGCU C GGGGCUC	607	AGCCAAU U UCUCGUG
115	CGGGGCU C UGUUCCC	608	GCCAAUU U CUCGUGC
119	GCUCUGU U CCCAGGA	609	CCAAUUU C UCGUGCC
120	CUCUGUU C CCAGGAC	611	AAUUUCU C GUGCCGC
146	CAGACAU C UGUGUCC	656	GAGCUGU U UGAGAAC
152	UCUGUGU C CCCCUCA	657	AGCUGUU U GAGAACA
158	UCCCCCU C AAAAGUC	668	AACACCU C GGCCCCC
165	CAAAAGU C AUCCUGC	677	GCCCCCU A CCAGCUC
168	AAGUCAU C CUGCCCC	684	ACCAGCU C CAGACCU
185	GGAGGCU C CGUGCUG	692	CAGACCU U UGUCCUG
209	AGCACCU C CUGUGAC	693	AGACCUU U GUCCUGC
227	CCCAAGU U GUUGGGC	696	CCUUUGU C CUGCCAG
230	AAGUUGU U GGGCAUA	709	AGCGACU C CCCCACA
237	UGGGCAU A GAGACCC	720	CACAACU U GUCAGCC
248	ACCCCGU U GCCUAAA	723	AACUUGU C AGCCCCC
253	GUUGCCU A AAAAGGA	735	CCCGGGU C CUAGAGG
263	AAGGAGU U GCUCCUG	738	GGGUCCU A GAGGUGG
267	AGUUGCU C CUGCCUG	765	CCGUGGU C UGUUCCC
293	AAGGUGU A UGAACUG	769	GGUCUGU U CCCUGGA
319	AGAAGAU A GCCAACC	770	GUCUGUU C CCUGGAC
335	AUGUGCU A UUCAAAC	785	GGGCUGU U CCCAGUC
337	GUGCUAU U CAAACUG	786	GGCUGUU C CCAGUCU
338	UGCUAUU C AAACUGC	792	UCCCAGU C UCGGAGG
359	GGGCAGU C AACAGCU	794	CCAGUCU C GGAGGCC
367	AACAGCU A AAACCUU	807	CCCAGGU C CACCUGG
374	AAAACCU U CCUCACC	833	CAGAGGU U GAACCCC
375	AAACCUU C CUCACCG	846	CCACAGU C ACCUAUG
378	CCUUCCU C ACCGUGU	851	GUCACCU A UGGCAAC

863	AACGACU C CUUCUCG	1408	UCGAGAU C UUGAGGG
866	GACUCCU U CUCGGCC	1410	GAGAUCU U GAGGGCA
867	ACUCCUU C UCGGCCA	1421	GGCACCU A CCUCUGU
869	UCCUUCU C GGCCAAG	1425	CCUACCU C UGUCGGG
881	AAGGCCU C AGUCAGU	1429	CCUCUGU C GGGCCAG
885	CCUCAGU C AGUGUGA	1444	GAGCACU C AAGGGGA
933	GUGCAGU A AUACUGG	1455	GGGAGGU C ACCCGCG
936	CAGUAAU A CUGGGGA	1482	AUGUGCU C UCCCCCC
978	UGACCAU C UACAGCU	1484	GUGCUCU C CCCCCGG
980	ACCAUCU A CAGCUUU	1493	CCCCGGU A UGAGAUU
986	UACAGCU U UCCGGCG	1500	AUGAGAU U GUCAUCA
987	ACAGCUU U CCGGCGC	1503	AGAUUGU C AUCAUCA
988	CAGCUUU C CGGCGCC	1506	UUGUCAU C AUCACUG
1005	ACGUGAU U CUGACGA	1509	UCAUCAU C ACUGUGG
1006	CGUGAUU C UGACGAA	1518	CUGUGGU A GCAGCCG
1023	CAGAGGU C UCAGAAG	1530	CCGCAGU C AUAAUGG
1025	GAGGUCU C AGAAGGG	1533	CAGUCAU A AUGGGCA
1066	CCACCCU A GAGCCAA	1551	CAGGCCU C AGCACGU
1092	AUGGGGU U CCAGCCC	1559	AGCACGU A CCUCUAU
1093	UGGGGUU C CAGCCCA	1563	CGUACCU C UAUAACC
1125	CCCAGCU C CUGCUGA	1565	UACCUCU A UAACCGC
1163	CGCAGCU U CUCCUGC	1567	CCUCUAU A ACCGCCA
1164	GCAGCUU C UCCUGCU	1584	GGAAGAU C AAGAAAU
1166	AGCUUCU C CUGCUCU	1592	AAGAAAU A CAGACUA
1172	UCCUGCU C UGCAACC	1599	ACAGACU A CAACAGG
1200	GCCAGCU U AUACACA	1651	CACGCCU C CCUGAAC
1201	CCAGCUU A UACACAA	1661	UGAACCU A UCCCGGG
1203	AGCUUAU A CACAAGA	1663	AACCUAU C CCGGGAC
1227	GGGAGCU U CGUGUCC	1678	AGGGCCU C UUCCUCG
1228	GGAGCUU C GUGUCCU	1680	GGCCUCU U CCUCGGC
1233	UUCGUGU C CUGUAUG	1681	GCCUCUU C CUCGGCC
1238	GUCCUGU A UGGCCCC	1684	UCUUCCU C GGCCUUC
1264	GAGGGAU U GUCCGGG	1690	UCGGCCU U CCCAUAU
1267	GGAUUGU C CGGGAAA	1691	CGGCCUU C CCAUAUU
1294	AGAAAAU U CCCAGCA	1696	UUCCCAU A UUGGUGG
1295	GAAAAUU C CCAGCAG	1698	CCCAUAU U GGUGGCA
1306	GCAGACU C CAAUGUG	1737	AAGACAU A UGCCAUG
1321	CCAGGCU U GGGGGAA	1750	UGCAGCU A CACCUAC
1334	AACCCAU U GCCCGAG	1756	UACACCU A CCGGCCC
1344	CCGAGCU C AAGUGUC	1787	AGGGCAU U GUCCUCA
1351	CAAGUGU C UAAAGGA	1790	GCAUUGU C CUCAGUC
1353	AGUGUCU A AAGGAUG	1793	UUGUCCU C AGUCAGA
1366	UGGCACU U UCCCACU	1797	CCUCAGU C AGAUACA
1367	GGCACUU U CCCACUG	1802	GUCAGAU A CAACAGC
1368	GCACUUU C CCACUGC	1812	ACAGCAU U UGGGGCC
1380	UGCCCAU C GGGGAAU	1813	CAGCAUU U GGGGCCA
1388	GGGGAAU C AGUGACU	1825	CCAUGGU A CCUGCAC
1398	UGACUGU C ACUCGAG	1837	CACACCU A AAACACU
1402	UGUCACU C GAGAUCU	1845	AAACACU A GGCCACG

1856	CACGCAU C UGAUCUG	2189	UAUUUAU U GAGUGUC
1861	AUCUGAU C UGUAGUC	2196	UGAGUGU C UUUUAUG
1865	GAUCUGU A GUCACAU	2198	AGUGUCU U UUAUGUA
1868	CUGUAGU C ACAUGAC	2199	GUGUCUU U UAUGUAG
1877	CAUGACU A AGCCAAG	2200	UGUCUUU U AUGUAGG
1901	CAAGACU C AAGACAU	2201	GUCUUUU A UGUAGGC
1912	ACAUGAU U GAUGGAU	2205	UUUAUGU A GGCUAAA
1922	UGGAUGU U AAAGUCU	2210	GUAGGCU A AAUGAAC
1923	GGAUGUU A AAGUCUA	2220	UGAACAU A GGCCUCU
1928	UUAAAGU C UAGCCUG	2224	CAUAGGU C UCUGGCC
1930	AAAGUCU A GCCUGAU	2226	UAGGUCU C UGGCCUC
1964	GAGACAU A GCCCCAC	2233	CUGGCCU C ACGGAGC
1983	AGGACAU A CAACUGG	2242	CGGAGCU C CCAGUCC
1996	GGGAAAU A CUGAAAC	2248	UCCCAGU C CAUGUCA
2005	UGAAACU U GCUGCCU	2254	UCCAUGU C ACAUUCA
2013	GCUGCCU A UUGGGUA	2259	GUCACAU U CAAGGUC
2015	UGCCUAU U GGGUAUG	2260	UCACAUU C AAGGUCA
2020	AUUGGGU A UGCUGAG	2266	UCAAGGU C ACCAGGU
2039	ACAGACU U ACAGAAG	2274	ACCAGGU A CAGUUGU
2040	CAGACUU A CAGAAGA	2279	GUACAGU U GUACAGG
2057	UGGCCCU C CAUAGAC	2282	CAGUUGU A CAGGUUG
2061	CCUCCAU A GACAUGU	2288	UACAGGU U GUACACU
2071	CAUGUGU A GCAUCAA	2291	AGGUUGU A CACUGCA
2076	GUAGCAU C AAAACAC	2321	AAAAGAU C AAAUGGG
2097	CCACACU U CCUGACG	2338	UGGGACU U CUCAUUG
2098	CACACUU C CUGACGG	2339	GGGACUU C UCAUUGG
2115	GCCAGCU U GGGCACU	2341	GACUUCU C AUUGGCC
2128	CUGCUGU C UACUGAC	2344	UUCUCAU U GGCCAAC
2130	GCUGUCU A CUGACCC	2358	CCUGCCU U UCCCCAG
2145	CAACCCU U GAUGAUA	2359	CUGCCUU U CCCCAGA
2152	UGAUGAU A UGUAUUU	2360	UGCCUUU C CCCAGAA
2156	GAUAUGU A UUUAUUC	2376	GAGUGAU U UUUCUAU
2158	UAUGUAU U UAUUCAU	2377	AGUGAUU U UUCUAUC
2159	AUGUAUU U AUUCAUU	2378	GUGAUUU U UCUAUCG
2160	UGUAUUU A UUCAUUU	2379	UGAUUUU U CUAUCGG
2162	UAUUUAU U CAUUUGU	2380	GAUUUUU C UAUCGGC
2163	AUUUAUU C AUUUGUU	2382	UUUUUCU A UCGGCAC
2166	UAUUCAU U UGUUAUU	2384	UUUCUAU C GGCACAA
2167	AUUCAUU U GUUAUUU	2399	AAGCACU A UAUGGAC
2170	CAUUUGU U AUUUUAC	2401	GCACUAU A UGGACUG
2171	AUUUGUU A UUUUACC	2411	GACUGGU A AUGGUUC
2173	UUGUUAU U UUACCAG	2417	UAAUGGU U CACAGGU
2174	UGUUAUU U UACCAGC	2418	AAUGGUU C ACAGGUU
2175	GUUAUUU U ACCAGCU	2425	CACAGGU U CAGAGAU
2176	UUAUUUU A CCAGCUA	2426	ACAGGUU C AGAGAUU
2183	ACCAGCU A UUUAUUG	2433	CAGAGAU U ACCCAGU
2185	CAGCUAU U UAUUGAG	2434	AGAGAUU A CCCAGUG
2186	AGCUAUU U AUUGAGU	2448	GAGGCCU U AUUCCUC
2187	GCUAUUU A UUGAGUG	2449	AGGCCUU A UUCCUCC

2451	GCCUUAU U CCUCCCU	2750	UAUGUGU A GACAAGC
2452	CCUUAUU C CUCCCUU	2759	ACAAGCU C UCGCUCU
2455	UAUUCCU C CCUUCCC	2761	AAGCUCU C GCUCUGU
2459	CCUCCCU U CCCCCCA	2765	UCUCGCU C UGUCACC
2460	CUCCCUU C CCCCCAA	2769	GCUCUGU C ACCCAGG
2479	GACACCU U UGUUAGC	2797	GUGCAAU C AUGGUUC
2480	ACACCUU U GUUAGCC	2803	UCAUGGU U CACUGCA
2483	CCUUUGU U AGCCACC	2804	CAUGGUU C ACUGCAG
2484	CUUUGUU A GCCACCU	2813	CUGCAGU C UUGACCU
2492	GCCACCU C CCCACCC	2815	GCAGUCU U GACCUUU
2504	CCCACAU A CAUUUCU	2821	UUGACCU U UUGGGCU
2508	CAUACAU U UCUGCCA	2822	UGACCUU U UGGGCUC
2509	AUACAUU U CUGCCAG	2823	GACCUUU U GGGCUCA
2510	UACAUUU C UGCCAGU	2829	UUGGGCU C AAGUGAU
2520	CCAGUGU U CACAAUG	2837	AAGUGAU C CUCCCAC
2521	CAGUGUU C ACAAUGA	2840	UGAUCCU C CCACCUC
2533	UGACACU C AGCGGUC	2847	CCCACCU C AGCCUCC
2540	CAGCGGU C AUGUCUG	2853	UCAGCCU C CUGAGUA
2545	GUCAUGU C UGGACAU	2860	CCUGAGU A GCUGGGA
2568	AGGGAAU A UGCCCAA	2872	GGACCAU A GGCUCAC
2579	CCAAGCU A UGCCUUG	2877	AUAGGCU C ACAACAC
2585	UAUGCCU U GUCCUCU	2899	GGCAAAU U UGAUUUU
2588	GCCUUGU C CUCUUGU	2900	GCAAAUU U GAUUUUU
2591	UUGUCCU C UUGUCCU	2904	AUUUGAU U UUUUUUU
2593	GUCCUCU U GUCCUGU	2905	UUUGAUU U UUUUUUU
2596	CUCUUGU C CUGUUUG	2906	UUGAUUU U UUUUUUU
2601	GUCCUGU U UGCAUUU	2907	UGAUUUU U UUUUUUU
2602	UCCUGUU U GCAUUUC	2908	GAUUUUU U UUUUUUU
2607	UUUGCAU U UCACUGG	2909	AUUUUUU U UUUUUUU
2608	UUGCAUU U CACUGGG	2910	UUUUUUU U UUUUUUU
2609	UGCAUUU C ACUGGGA	2911	UUUUUUU U UUUUUUU
2620	GGGAGCU U GCACUAU	2912	UUUUUUU U UUUUUUC
2626	UUGCACU A UUGCAGC	2913	UUUUUUU U UUUUUCA
2628	GCACUAU U GCAGCUC	2914	UUUUUUU U UUUUCAG
2635	UGCAGCU C CAGUUUC	2915	UUUUUUU U UUUCAGA
2640	CUCCAGU U UCCUGCA	2916	UUUUUUU U UUCAGAG
2641	UCCAGUU U CCUGCAG	2917	UUUUUUU U UCAGAGA
2642	CCAGUUU C CUGCAGU	2918	UUUUUUU U CAGAGAC
2653	CAGUGAU C AGGGUCC	2919	UUUUUUU C AGAGACG
2659	UCAGGGU C CUGCAAG	2931	ACGGGGU C UCGCAAC
2689	CCAAGGU A UUGGAGG	2933	GGGGUCU C GCAACAU
2691	AAGGUAU U GGAGGAC	2941	GCAACAU U GCCCAGA
2700	GAGGACU C CCUCCCA	2951	CCAGACU U CCUUUGU
2704	ACUCCCU C CCAGCUU	2952	CAGACUU C CUUUGUG
2711	CCCAGCU U UGGAAGG	2955	ACUUCCU U UGUGUUA
2712	CCAGCUU U GGAAGGG	2956	CUUCCUU U GUGUUAG
2721	GAAGGGU C AUCCGCG	2961	UUUGUGU U AGUUAAU
2724	GGGUCAU C CGCGUGU	2962	UUGUGUU A GUUAAUA
2744	UGUGUGU A UGUGUAG	2965	UGUUAGU U AAUAAAG

2966	GUUAGUU A AUAAAGC
2969	AGUUAAU A AAGCUUU
2975	UAAAGCU U UCUCAAC
2976	AAAGCUU U CUCAACU
2977	AAGCUUU C UCAACUG
2979	GCUUUCU C AACUGCC

Table 3

Mouse ICAM HH Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
11	CCCugGU C acCGuUG	367	AAugGCU u cAACCcg
23	CaGuGgU u CUCUGCU	374	gAAgCCU U CCUgcCC
26	uGgUuCU C UGCUcCU	375	AAgCCUU C CUgcCCc
31	CUCUGCU c CUCcaca	378	CuacCaU C ACCGUGU
34	UuCUcaU a AGgGUcG	386	ACCGUGU A uUcGuuU
40	gCAcAcU U GuAgCCU	394	CcGGACU u ucGAuCu
48	aggACCU C AGCCUgG	420	CACaCuU C CCCcCcg
54	UggGCCU C GugAUGG	425	CaCCCCU C ccaGCAG
58	CaUgcCU u UaGCUCC	427	CagCUCU c aGCAGug
64	cAcccCU C CCAGCAG	450	AGgACCU c ACCCUgC
96	CucugCU C CUGGcCC	451	GAAaCcU u uCCUuuG
102	UgCcaGU a CUGCUgG	456	UUACCCU c aGCcaCu
108	cuCUGCU C cuGGCcC	495	CuAcCaU C ACCGUGu
115	uGGuuCU C UGcUCCu	510	UGCUGCU C CGUGGGG
119	GgaaUGU c aCCAGGA	564	CUcAGGU a uCcAuCc
120	CUCUGcU C CugGccC	592	GAaAGAU C ACaugGG
146	CAGuCgU C cGcuUCC	607	AGCCAAU U UCUCaUG
152	UCUGUGU C agCCaCu	608	GCCAAUU U CUCaUGC
158	UCCuguU u AAAAacC	609	CCAAUUU C UCaUGCC
165	CAgAAGU u gUuuUGC	611	AAUUUCU C aUGCCGC
168	AAGcCuU C CUGCCCC	656	aAGCUGU U UGAGcug
185	GGuGGgU C CGUGCaG	657	AGCUGUU U GAGcugA
209	gcCACuU C CUcUGgC	668	cgagCCU a GGCCaCC
227	CagAAGU U GUUuuGC	677	GaCCuCU A CCAGCcu
230	AAGUUGU U uuGCucc	684	uuCAGCU C CgGuCCU
237	UGuGCuU u GAGAaCu	692	CgGACuU U cGauCUu
248	AaCCCaU c uCCUAAA	693	AGgaCcU c acCCUGC
253	ccUGCCU A AggAaGA	696	CCUgUuU C CUGCCuc
263	AgGGuuU c uCUaCUG	709	gGCGgCU C CaCCuCA
267	AGggGCU C CUGCCUa	720	uACAACU U uUCAGCu
293	AAGcUGU u UGAgCUG	723	AACUUuU C AGCuCCg
319	AGgAGAU A cugAgCC	735	aCCaGaU C CUgGAGa
335	cUGUGCU u UgagAAC	738	uGGgCCU c GuGaUGG
337	GUcCaAU U CAcACUG	765	CaGUcGU C cGcUuCC
338	aGCUgUU u gAgCUGa	769	GGcCUGU U uCCUGcc
359	GuGCAGU C guCcGCU	770	uUuUGcU C CCUGGAa
785	GGcCUGU U uCCuGcC	1353	AGUGggU c gAaGgUG
786	GcCUGUU u CCuGcCU	1366	UaaCAgU c UaCaACU
792	UggagGU C UCGGAaG	1367	aGCACcU c CCCACcu
794	CugGgCU u GGAGaCu	1368	GuACUgU a CCACUcu
807	CuCgGaU a uACCUGG	1380	UGCCCAU C GGGGugg
833	CAaAGcU c GAcaCCC	1388	GGaGAcU C AGUGgCU
846	CCcugGU C ACCguUG	1398	UGgCUGU C ACagaAc
851	GagACCU c UacCAgC	1402	UGUgcuU u GAGAaCU

863	AgCcACU u CcUCUgG	1408	gCGAGAU C ggGgaGG
866	GAagCCU U CcuGcCC	1410	GAGgUCU c GgaaGgg
867	AuUCgUU u cCGGagA	1421	ccCACCU A CuUuUGU
869	UCuUcCU C augCAAG	1425	aCUgCCU u gGUaGaG
881	AuGGCuU C AacCcGU	1429	uCUCUaU u GccCCuG
885	CCUugGU a gagGUGA	1444	GAaggCU C AgGaGGA
933	cUauAaU c AUuCUGG	1455	GGaAuGU C ACCaGga
936	uAaUcAU u CUGGuGc	1482	AguUGuU u UgCuCCC
978	UaACagU C UACAaCU	1484	cUGuUCU u CCuCauG
980	ACagUCU A CAaCUUU	1493	CuguGcU u UGAGAac
986	UACAaCU U UuCaGCu	1500	AUGAaAU c aUggUCc
987	ACAaCUU U uCaGCuC	1503	gGAcUaU a AUCAUuc
988	CAaCUUU u CaGCuCC	1506	UUaUguU u AUaACcG
1005	ACcaGAU c CUGgaGA	1509	cuAcCAU C ACcGUGu
1006	uGaGAgU C UGggGAA	1518	ucaUGGU c cCAGgCG
1023	ugGAGGU C UCgGAAG	1530	CuauAaU C AUucUGG
1025	GAGGUCU C gGAAGGG	1533	ugGUCAU u gUGGGCc
1066	CCACuCU c aAaauAA	1551	CAuGCCU u AGCAgcU
1092	AcuGGaU c uCAGgCC	1559	AGCACcU c CCcaccU
1093	UGGaccU u CAGCCaA	1563	CuUAugU u UAUAACC
1125	CCCAaCU C uUcuUGA	1565	UAugUuU A UAACCGC
1163	CGaAGCU U CUuuUGC	1567	ugUuUAU A ACCGCCA
1164	GaAGCUU C UuuUGCU	1584	GaAAGAU C AgGAuAU
1166	AGCUUCU u uUGCUCU	1592	AgGAuAU A CAaguUA
1172	UCCUGuU u aaaAACC	1599	ACAaguU A CAgaAGG
1200	cuCuGCU c cUcCACA	1651	CcCaCCU C CCUGAgC
1201	gCuGCUU u UgaACAg	1661	gaAACCU u UCCuuuG
1203	AcuUUuU u CACcAGu	1663	AACCUuU C CuuuGAa
1227	GGuAcaU a CGUGUgC	1678	AGGaCCU C agCCUgG
1228	GaAGCUU C uUuUgCU	1680	aGCCaCU U CCUCuGg
1233	UUCGUuU C CgGagaG	1681	GCCaCUU C CUCuGgC
1238	GUgCUGU A UGGuCCu	1684	aCUUCCU C uGgCUgu
1264	GAaGGgU c GUgCaaG	1690	cCGGaCU U uCgAUcU
1267	uGAgaGU C uGGGgAA	1691	CGGaCUU u CgAUcUU
1294	AGgAgAU a CugAGCc	1696	UgCCCAU c ggGGUGG
1295	GAggggU C uCAGCAG	1698	CggAUAU a ccUGGag
1306	GCAGACU C ugAaaUG	1737	gAGACcU c UaCCAgc
1321	gaAGGCU c aGGaGgA	1750	gGCgGCU c CACCUca
1334	AACCCAU c uCCuaAa	1756	gAagCCU u CCuGCCC
1344	auGAGCU C gAGaGUg	1787	gaGaCAU U GUCCcCA
1351	ugAaUGU a UAAguuA	1790	GCAUUGU u CUCuaau
1793	UgGUCCU C gGcugGA	2173	UUagagU U UUACCAG
1797	CacCAGU C AcAUAaA	2174	UagagUU U UACCAGC
1802	acCAGAU c CuggAGa	2175	agagUUU U ACCAGCU
1812	ACuGgAU c UcaGGCC	2176	gagUUUU A CCAGCUA
1813	CAGCAUU U acccuCA	2183	ACCAGCU A UUUAUUG
1825	CCAcGcU A CCUcugC	2185	CAGCUAU U UAUUGAG
1837	CAugCCU u uAgCuCc	2186	AGCUAUU U AUUGAGU
1845	cgAgcCU A GGCCACc	2187	GCUAUUU A UUGAGUa

1856	CggaCuU u cGAUCUu	2189	UAUUUAU U GAGUacC
1861	AcaUGAU a UccAGUa	2196	caAcUcU u cUUgAUG
1865	cAcuUGU A GcCuCAg	2198	gcaGcCU c UUAUGUu
1868	CaccAGU C ACAUaAa	2199	GccUCUU a UgUuUAu
1877	CAUGcCU u AGCagcu	2200	UcUuccU c AUGcAaG
1901	uAAaACU C AAGggAc	2201	aagUUUU A UGUcGGC
1912	AuAUagU a GAUcagU	2205	UUUAUGU c GGCcugA
1922	UGaAUGU a uAAGUua	2210	GgAGaCU c AgUGgcu
1923	uGAUGcU c AgGUaUc	2220	cuggCAU u GuCCUCU
1928	UUAgAGU u UuaCCaG	2224	CucAGGU a UCcauCC
1930	AgAGUuU u aCCaGcU	2226	UgGaUCU C aGGCCgC
1964	GAGACAU u GuCCCca	2233	CUGaCCU C cuGGAGg
1983	AGGAuAU A CAAgUua	2242	uGGAGCU a gCgGaCC
1996	aGGAgAU A CUGAgcC	2248	UauCcaU C CAUccCA
2005	UGgAgCU a GCgGaCc	2254	UCCAauU C ACAcUgA
2013	GCUauuU A UUGaGUA	2259	aUCACAU U CAcGGUg
2015	UGCCcAU c GGGgugG	2260	UCACAUU C AcGGUgc
2020	ggUGGuU c UuCUGAG	2266	ggAAuGU C ACCAGGa
2039	gCuGgCU a gCAGAgG	2274	ACCAGaU c CuGgaGa
2040	CuGACcU c CuGgAGg	2279	GaAggGU c GUgCAaG
2057	UGcuCCU C CAcAucC	2282	aAGcUGU u ugaGcUG
2061	CuaCCAU c acCgUGU	2288	UAuAaGU U aUggcCU
2071	CAcuUGU A GCcUCAg	2291	caGUgGU u CuCUGCu
2076	GUAGCcU C AgAgCua	2321	gAAAGAU C AcAUGGG
2097	CaACuCU U CuUGAuG	2338	UGaGACU c CUgccUG
2098	CACACUU C CcccCcG	2339	GaaACcU u UCcUUuG
2115	GCCAGCU c GGaggaU	2341	GACcUCU a ccaGcCu
2128	CaGCUaU u UAuUGAg	2344	UUucgAU c uuCCAgC
2130	cCUGUuU c CUGcCuC	2358	CCcagCU c UCagCAG
2145	CAACuCU U cuUGAUg	2359	CUGCuUU U gaaCAGA
2152	UauUaAU u UagAgUU	2360	aaCCUUU C CuuuGAA
2156	uugAUGU A UUUAUUa	2376	agGUGgU U cUUCUga
2158	gAUGUAU U UAUUaAU	2377	gGUGgUU c UUCUgag
2159	AUGUAUU U AUUaAUU	2378	agGgUUU c UCUAcuG
2160	UGUAUUU A UUaAUUU	2379	UGcUUUU c ucAUaaG
2162	UAUUUAU U aAUUUag	2380	aAgUUUU a UgUCGGC
2163	AUgUAUU u AUUaaUU	2382	aUUcUCU A UuGcCcC
2166	acUUCAU U cucUAUU	2384	aUcCagU a GaCACAA
2167	AUguAUU U aUUAaUU	2399	AAaCACU A UgUGGAC
2170	uAUUUaU U AaUUUAg	2401	aagCUgU u UGagCUG
2171	AgUUGUU u UgcUcCC	2411	uACUGGU c AgGaUgC
2417	gAAUGGU a CAuAcGU	2691	AAuGUcU c cGAGGcC
2418	AcUGGaU C uCAGGcc	2700	GAaGcCU u CCUgCCc
2425	CAugGGU c gAGgGuU	2704	gacCuCU a CCAGCcU
2426	AuuaaUU u AGAGuUU	2711	CCCAGCU c UcagcaG
2433	uAGAGuU U uaCCAGc	2712	gagGucU c GGAAGGG
2434	AGAGuUU u aCCAGcu	2721	GuAGGGU C gUgCaaG
2448	GAaGCCU U ccUgCcC	2724	GGuaCAU a CGuGUGc
2449	AaGCCUU c cUgCcCC	2744	gGUGgGU c cGUGcAG

2451	GCCUguU U CCUgCCU
2452	CCUguUU C CUgCCUc
2455	gAagCCU u CCUgCCC
2459	CCaCaCU U CCCCCCc
2460	CaCaCUU C CCCCCcg
2479	GAgACCU c UaccAGC
2480	uCACCgU U GUgAuCC
2483	CCaaUGU c AGCCACC
2484	CUUUuUU c aCCAguc
2492	agCACCU C CCCACCu
2504	CCCACcU A CuUUUgU
2508	uAUcCAU c caUcCCA
2509	uUAgAgU U uUaCCAG
2510	UAgAgUU u UaCCAGc
2520	CuuuUGU U CcCAAUG
2521	CAGcaUU u ACccUcA
2533	UGAugCU C AGguaUC
2540	CAGCaGU C cgcUgUG
2545	GUgcUGU a UGGuCcU
2568	guGaAgU c UGuCaAA
2579	auAAGuU A UGgCcUG
2585	cugGCaU U GUuCUCU
2588	GCaUUGU u CUCUaaU
2591	UgGUuCU C UgcUCCU
2593	cUuCUuU U GcuCUGc
2596	CUuUUGU u CccaaUG
2601	acCgUGU a UuCgUUU
2602	UCCaGcU a cCAUccC
2607	cUcGgAU a UacCUGG
2608	caGCAgU c CgCUGuG
2609	gGaAUgU C ACcaGGA
2620	aGGAcCU c aCcCUgc
2626	UUuCgaU c UUcCAGC
2628	GCACacU U GuAGCcu
2635	UuCAGCU C CgGUccu
2640	ggCCuGU U UCCUGCc
2641	cCCAGcU c uCaGCAG
2642	CCuGUUU C CUGCcuc
2653	uAcUGgU C AGGaUgC
2659	gaAGGGU C gUGCAAG
2689	CuAAuGU c UccGAGG
2750	UAUuUaU u GAguAcC
2759	cCggaCU u UCGaUCU
2761	AgGacCU C aCcCUGc
2765	UuUuGCU C UGcCgCu
2769	agUCUGU C AaaCAGG
2797	aUGaAAU C AUGGUcC
2803	UCAUGGU c CcagGCg
2804	ggUGGgU C cgUGCAG
2813	CUcCgGU C cUGACCc
2815	aCAGUCU a cAaCUUU
2821	cUGACCU c cUGGagg
2822	gGAgCcU c cGGaCUu
2823	ugCCUUU a GcuCcCA
2829	cUGGaCU a uAaUcAU
2837	AgGUGgU u CUuCuga
2840	UGAgaCU C CugCCUg
2847	CCaAugU C AGCCaCC
2853	gCAGCCU C uUauGUu
2860	gCcaAGU A aCUGuGA
2872	GGACCuU c aGCcaAg
2877	uUccGCU a cCAuCAC
2899	cGgAcuU U cGAUcUU
2900	uuAAuUU a GAgUUUU
2904	AcUUcAU U cUcUaUU
2905	cUUcAUU c UcUaUUg
2906	UUGAUgU a UUUaUUa
2907	UGuaUUU a UUaaUUU
2908	GAagcUU c UUUUgcU
2909	AgcUUcU U UUgcUcU
2910	UgUaUUU a UUaaUUU
2911	UgUaUUU a UUaaUUU
2912	UUgUUcU c UaaUgUC
2913	UUUcUcU a cUggUCA
2914	UgcUUUU c UcaUaAG
2915	aUUUaUU a aUUuAGA
2916	UaUUcgU U UcCgGAG
2917	aUUcgUU U cCgGAGA
2918	UUcgUUU c CgGAGAg
2919	UUcUcaU a AGgGuCG
2931	ugGaGGU C UCGgAAg
2933	GaGGUCU C GgAAggg
2941	GagACAU U GuCCccA
2951	CCAcgCU a CCUcUGc
2952	CAGcagU C CgcUGUG
2955	AgUgaCU c UGUGUcA
2956	uUUCCUU U GaaUcAa
2961	UcUGUGU c AGccAcU
2962	aUGUaUU u aUUAAUu
2965	UuUgAaU c AAUAAAG

2966	GcUgGcU A gcAgAGg
2969	AaUcAAU A AAGuUUU
2975	UAgAGuU U UacCAgC
2976	gAgGgUU U CUCuACU
2977	AAGCUgU u UgAgCUG
2979	uCaUUCU C uAuUGCC

Table 4
Human ICAM HH Ribozyme Sequences

nt. Position	Ribozyme Sequence
11	CAGCGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
26	AGUAGCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
31	CUCUGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CAACUCU CUGAUGAGGCCGAAAGGCCGAA AGUAGCA
40	AGGUUGC CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
48	CGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUGC
54	CCAUAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
58	GGAGCCA CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
64	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAUA
96	GGACCAG CUGAUGAGGCCGAAAGGCCGAA AGUGCGG
102	CGAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
108	GAGCCCC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
115	GGGAACA CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
119	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
120	GUCCUGG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
146	GGACACA CUGAUGAGGCCGAAAGGCCGAA AUGUCUG
152	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GACUUUU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
165	GCAGGAU CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGACUU
185	CAGCACG CUGAUGAGGCCGAAAGGCCGAA AGCCUCC
209	GUCACAG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
227	GCCCAAC CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
230	UAUGCCC CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237	GGGUCUC CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
248	UUUAGGC CUGAUGAGGCCGAAAGGCCGAA ACGGGGU
253	UCCUUUU CUGAUGAGGCCGAAAGGCCGAA AGGCAAC
263	CAGGAGC CUGAUGAGGCCGAAAGGCCGAA ACUCCUU
267	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
293	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA ACACCUU
319	GGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCUUCU
335	GUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
337	CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGCAC
338	GCAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGCA
359	AGCUGUU CUGAUGAGGCCGAAAGGCCGAA ACUGCCC
367	AAGGUUU CUGAUGAGGCCGAAAGGCCGAA AGCUGUU
374	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
375	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
386	AGUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
420	AAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
425	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AGGGGAG

427	GGCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGG
450	GUAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCU
451	CGUAGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUC
456	GGCAGCG	CUGAUGAGGCCGAAAGGCCGAA	AGGGUAA
495	CCACGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGG
510	CCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
564	UGGUCGU	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAG
592	CCAUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUC
607	CACGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
608	GCACGAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
609	GGCACGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
611	GCGGCAC	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
656	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUC
657	UGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
668	GGGGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUU
677	GAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGC
684	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
692	CAGGACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
693	GCAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
696	CUGGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAGG
709	UGUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGCU
720	GGCUGAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUG
723	GGGGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUU
735	CCUCUAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCGGG
738	CCACCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGACCC
765	GGGAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCACGG
769	UCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGACC
770	GUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGAC
785	GACUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCC
786	AGACUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCC
792	CCUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGA
794	GGCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACUGG
807	CCAGGUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGGG
833	GGGGUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCUCUG
846	CAUAGGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGG
851	GUUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAC
863	CGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUU
866	GGCCGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGUC
867	UGGCCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGU
869	CUUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGAAGGA
881	ACUGACU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUU
885	UCACACU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGG
933	CCAGUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAC
936	UCCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUACUG
978	AGCUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUCA
980	AAAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGU
986	CGCCGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUA
987	GCGCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
988	GGCGCCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCUG

1005	UCGUCAG CUGAUGAGGCCGAAAGGCCGAA AUCACGU
1006	UUCGUCA CUGAUGAGGCCGAAAGGCCGAA AAUCACG
1023	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACCUCUG
1025	CCCUUCU CUGAUGAGGCCGAAAGGCCGAA AGACCUC
1066	UUGGCUC CUGAUGAGGCCGAAAGGCCGAA AGGGUGG
1092	GGGCUGG CUGAUGAGGCCGAAAGGCCGAA ACCCCAU
1093	UGGGCUG CUGAUGAGGCCGAAAGGCCGAA AACCCCA
1125	UCAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
1163	GCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGCUGCG
1164	AGCAGGA CUGAUGAGGCCGAAAGGCCGAA AAGCUGC
1166	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCU
1172	GGUUGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
1200	UGUGUAU CUGAUGAGGCCGAAAGGCCGAA AGCUGGC
1201	UUGUGUA CUGAUGAGGCCGAAAGGCCGAA AAGCUGG
1203	UCUUGUG CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
1227	GGACACG CUGAUGAGGCCGAAAGGCCGAA AGCUCCC
1228	AGGACAC CUGAUGAGGCCGAAAGGCCGAA AAGCUCC
1233	CAUACAG CUGAUGAGGCCGAAAGGCCGAA ACACGAA
1238	GGGGCCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAC
1264	CCCGGAC CUGAUGAGGCCGAAAGGCCGAA AUCCCUC
1267	UUUCCCG CUGAUGAGGCCGAAAGGCCGAA ACAAUCC
1294	UGCUGGG CUGAUGAGGCCGAAAGGCCGAA AUUUUCU
1295	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AAUUUUC
1306	CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGUCUGC
1321	UUCCCCC CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
1334	CUCGGGC CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
1344	GACACUU CUGAUGAGGCCGAAAGGCCGAA AGCUCGG
1351	UCCUUUA CUGAUGAGGCCGAAAGGCCGAA ACACUUG
1353	CAUCCUU CUGAUGAGGCCGAAAGGCCGAA AGACACU
1366	AGUGGGA CUGAUGAGGCCGAAAGGCCGAA AGUGCCA
1367	CAGUGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGCC
1368	GCAGUGG CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
1380	AUUCCCC CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
1388	AGUCACU CUGAUGAGGCCGAAAGGCCGAA AUUCCCC
1398	CUCGAGU CUGAUGAGGCCGAAAGGCCGAA ACAGUCA
1402	AGAUCUC CUGAUGAGGCCGAAAGGCCGAA AGUGACA
1408	CCCUCAA CUGAUGAGGCCGAAAGGCCGAA AUCUCGA
1410	UGCCCUC CUGAUGAGGCCGAAAGGCCGAA AGAUCUC
1421	ACAGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
1425	CCCGACA CUGAUGAGGCCGAAAGGCCGAA AGGUAGG
1429	CUGGCCC CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
1444	UCCCCUU CUGAUGAGGCCGAAAGGCCGAA AGUGCUC
1455	CGCGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUCCC
1482	GGGGGGA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
1484	CCGGGGG CUGAUGAGGCCGAAAGGCCGAA AGAGCAC
1493	AAUCUCA CUGAUGAGGCCGAAAGGCCGAA ACCGGGG
1500	UGAUGAC CUGAUGAGGCCGAAAGGCCGAA AUCUCAU
1503	UGAUGAU CUGAUGAGGCCGAAAGGCCGAA ACAAUCU
1506	CAGUGAU CUGAUGAGGCCGAAAGGCCGAA AUGACAA

1509	CCACAGU CUGAUGAGGCCGAAAGGCCGAA AUGAUGA
1518	CGGCUGC CUGAUGAGGCCGAAAGGCCGAA ACCACAG
1530	CCAUUAU CUGAUGAGGCCGAAAGGCCGAA ACUGCGG
1533	UGCCCAU CUGAUGAGGCCGAAAGGCCGAA AUGACUG
1551	ACGUGCU CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
1559	AUAGAGG CUGAUGAGGCCGAAAGGCCGAA ACGUGCU
1563	GGUUAUA CUGAUGAGGCCGAAAGGCCGAA AGGUACG
1565	GCGGUUA CUGAUGAGGCCGAAAGGCCGAA AGAGGUA
1567	UGGCGGU CUGAUGAGGCCGAAAGGCCGAA AUAGAGG
1584	AUUUCUU CUGAUGAGGCCGAAAGGCCGAA AUCUUCC
1592	UAGUCUG CUGAUGAGGCCGAAAGGCCGAA AUUUCUU
1599	CCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGUCUGU
1651	GUUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCGUG
1661	CCCGGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUCA
1663	GUCCCGG CUGAUGAGGCCGAAAGGCCGAA AUAGGUU
1678	CGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGGCCCU
1680	GCCGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGGCC
1681	GGCCGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
1684	GAAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
1690	AUAUGGG CUGAUGAGGCCGAAAGGCCGAA AGGCCGA
1691	AAUAUGG CUGAUGAGGCCGAAAGGCCGAA AAGGCCG
1696	CCACCAA CUGAUGAGGCCGAAAGGCCGAA AUGGGAA
1698	UGCCACC CUGAUGAGGCCGAAAGGCCGAA AUAUGGG
1737	CAUGGCA CUGAUGAGGCCGAAAGGCCGAA AUGUCUU
1750	GUAGGUG CUGAUGAGGCCGAAAGGCCGAA AGCUGCA
1756	GGGCCGG CUGAUGAGGCCGAAAGGCCGAA AGGUGUA
1787	UGAGGAC CUGAUGAGGCCGAAAGGCCGAA AUGCCCU
1790	GACUGAG CUGAUGAGGCCGAAAGGCCGAA ACAAUGC
1793	UCUGACU CUGAUGAGGCCGAAAGGCCGAA AGGACAA
1797	UGUAUCU CUGAUGAGGCCGAAAGGCCGAA ACUGAGG
1802	GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AUCUGAC
1812	GGCCCCA CUGAUGAGGCCGAAAGGCCGAA AUGCUGU
1813	UGGCCCC CUGAUGAGGCCGAAAGGCCGAA AAUGCUG
1825	GUGCAGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
1837	AGUGUUU CUGAUGAGGCCGAAAGGCCGAA AGGUGUG
1845	CGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
1856	CAGAUCA CUGAUGAGGCCGAAAGGCCGAA AUGCGUG
1861	GACUACA CUGAUGAGGCCGAAAGGCCGAA AUCAGAU
1865	AUGUGAC CUGAUGAGGCCGAAAGGCCGAA ACAGAUC
1868	GUCAUGU CUGAUGAGGCCGAAAGGCCGAA ACUACAG
1877	CUUGGCU CUGAUGAGGCCGAAAGGCCGAA AGUCAUG
1901	AUGUCUU CUGAUGAGGCCGAAAGGCCGAA AGUCUUG
1912	AUCCAUC CUGAUGAGGCCGAAAGGCCGAA AUCAUGU
1922	AGACUUU CUGAUGAGGCCGAAAGGCCGAA ACAUCCA
1923	UAGACUU CUGAUGAGGCCGAAAGGCCGAA AACAUCC
1928	CAGGCUA CUGAUGAGGCCGAAAGGCCGAA ACUUUAA
1930	AUCAGGC CUGAUGAGGCCGAAAGGCCGAA AGACUUU
1964	GUGGGGC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
1983	CCAGUUG CUGAUGAGGCCGAAAGGCCGAA AUGUCCU

1996	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCCC
2005	AGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCA
2013	UACCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
2015	CAUACCC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGCA
2020	CUCAGCA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAU
2039	CUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGU
2040	UCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUG
2057	GUCUAUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCA
2061	ACAUGUC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGG
2071	UUGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACACAUG
2076	GUGUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUAC
2097	CGUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGG
2098	CCGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AGUGCCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	GUCAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAG
2130	GGGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
2145	UAUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AGGGUUG
2152	AAAUACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUCA
2156	GAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAUC
2158	AUGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUA
2159	AAUGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2160	AAAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	ACAAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2163	AACAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
2166	AAUAACA	CUGAUGAGGCCGAAAGGCCGAA	AUGAAUA
2167	AAAUAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
2170	GUAAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAAUG
2171	GGUAAAA	CUGAUGAGGCCGAAAGGCCGAA	AACAAAU
2173	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	AUAACAA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAUAACA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAC
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUAA
2183	CAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2186	ACUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGCU
2187	CACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GACACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2196	CAUAAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACUCA
2198	UACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGACACU
2199	CUACAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGACAC
2200	CCUACAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACA
2201	GCCUACA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAC
2205	UUUAGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAA
2210	GUUCAUU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUAC
2220	AGAGACC	CUGAUGAGGCCGAAAGGCCGAA	AUGUUCA
2224	GGCCAGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUAUG
2226	GAGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGACCUA
2233	GCUCCGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
2242	GGACUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCG

2248	UGACAUG CUGAUGAGGCCGAAAGGCCGAA ACUGGGA
2254	UGAAUGU CUGAUGAGGCCGAAAGGCCGAA ACAUGGA
2259	GACCUUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAC
2260	UGACCUU CUGAUGAGGCCGAAAGGCCGAA AAUGUGA
2266	ACCUGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGA
2274	ACAACUG CUGAUGAGGCCGAAAGGCCGAA ACCUGGU
2279	CCUGUAC CUGAUGAGGCCGAAAGGCCGAA ACUGUAC
2282	CAACCUG CUGAUGAGGCCGAAAGGCCGAA ACAACUG
2288	AGUGUAC CUGAUGAGGCCGAAAGGCCGAA ACCUGUA
2291	UGCAGUG CUGAUGAGGCCGAAAGGCCGAA ACAACCU
2321	CCCAUUU CUGAUGAGGCCGAAAGGCCGAA AUCUUUU
2338	CAAUGAG CUGAUGAGGCCGAAAGGCCGAA AGUCCCA
2339	CCAAUGA CUGAUGAGGCCGAAAGGCCGAA AAGUCCC
2341	GGCCAAU CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
2344	GUUGGCC CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
2358	CUGGGGA CUGAUGAGGCCGAAAGGCCGAA AGGCAGG
2359	UCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCAG
2360	UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGGCA
2376	AUAGAAA CUGAUGAGGCCGAAAGGCCGAA AUCACUC
2377	GAUAGAA CUGAUGAGGCCGAAAGGCCGAA AAUCACU
2378	CGAUAGA CUGAUGAGGCCGAAAGGCCGAA AAAUCAC
2379	CCGAUAG CUGAUGAGGCCGAAAGGCCGAA AAAAUCA
2380	GCCGAUA CUGAUGAGGCCGAAAGGCCGAA AAAAAUC
2382	GUGCCGA CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
2384	UUGUGCC CUGAUGAGGCCGAAAGGCCGAA AUAGAAA
2399	GUCCAUA CUGAUGAGGCCGAAAGGCCGAA AGUGCUU
2401	CAGUCCA CUGAUGAGGCCGAAAGGCCGAA AUAGUGC
2411	GAACCAU CUGAUGAGGCCGAAAGGCCGAA ACCAGUC
2417	ACCUGUG CUGAUGAGGCCGAAAGGCCGAA ACCAUUA
2418	AACCUGU CUGAUGAGGCCGAAAGGCCGAA AACCAUU
2425	AUCUCUG CUGAUGAGGCCGAAAGGCCGAA ACCUGUG
2426	AAUCUCU CUGAUGAGGCCGAAAGGCCGAA AACCUGU
2433	ACUGGGU CUGAUGAGGCCGAAAGGCCGAA AUCUCUG
2434	CACUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUCU
2448	GAGGAAU CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
2449	GGAGGAA CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
2451	AGGGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAGGC
2452	AAGGGAG CUGAUGAGGCCGAAAGGCCGAA AAUAAGG
2455	GGGAAGG CUGAUGAGGCCGAAAGGCCGAA AGGAAUA
2459	UGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGGGAGG
2460	UUGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGGAG
2479	GCUAACA CUGAUGAGGCCGAAAGGCCGAA AGGUGUC
2480	GGCUAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUGU
2483	GGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAAAGG
2484	AGGUGGC CUGAUGAGGCCGAAAGGCCGAA AACAAAG
2492	GGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGC
2504	AGAAAUG CUGAUGAGGCCGAAAGGCCGAA AUGUGGG
2508	UGGCAGA CUGAUGAGGCCGAAAGGCCGAA AUGUAUG
2509	CUGGCAG CUGAUGAGGCCGAAAGGCCGAA AAUGUAU

2510	ACUGGCA CUGAUGAGGCCGAAAGGCCGAA AAAUGUA
2520	CAUUGUG CUGAUGAGGCCGAAAGGCCGAA ACACUGG
2521	UCAUUGU CUGAUGAGGCCGAAAGGCCGAA AACACUG
2533	GACCGCU CUGAUGAGGCCGAAAGGCCGAA AGUGUCA
2540	CAGACAU CUGAUGAGGCCGAAAGGCCGAA ACCGCUG
2545	AUGUCCA CUGAUGAGGCCGAAAGGCCGAA ACAUGAC
2568	UUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUUCCCU
2579	CAAGGCA CUGAUGAGGCCGAAAGGCCGAA AGCUUGG
2585	AGAGGAC CUGAUGAGGCCGAAAGGCCGAA AGGCAUA
2588	ACAAGAG CUGAUGAGGCCGAAAGGCCGAA ACAAGGC
2591	AGGACAA CUGAUGAGGCCGAAAGGCCGAA AGGACAA
2593	ACAGGAC CUGAUGAGGCCGAAAGGCCGAA AGAGGAC
2596	CAAACAG CUGAUGAGGCCGAAAGGCCGAA ACAAGAG
2601	AAAUGCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAC
2602	GAAAUGC CUGAUGAGGCCGAAAGGCCGAA AACAGGA
2607	CCAGUGA CUGAUGAGGCCGAAAGGCCGAA AUGCAAA
2608	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AAUGCAA
2609	UCCCAGU CUGAUGAGGCCGAAAGGCCGAA AAAUGCA
2620	AUAGUGC CUGAUGAGGCCGAAAGGCCGAA AGCUCCC
2626	GCUGCAA CUGAUGAGGCCGAAAGGCCGAA AGUGCAA
2628	GAGCUGC CUGAUGAGGCCGAAAGGCCGAA AUAGUGC
2635	GAAACUG CUGAUGAGGCCGAAAGGCCGAA AGCUGCA
2640	UGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACUGGAG
2641	CUGCAGG CUGAUGAGGCCGAAAGGCCGAA AACUGGA
2642	ACUGCAG CUGAUGAGGCCGAAAGGCCGAA AAACUGG
2653	GGACCCU CUGAUGAGGCCGAAAGGCCGAA AUCACUG
2659	CUUGCAG CUGAUGAGGCCGAAAGGCCGAA ACCCUGA
2689	CCUCCAA CUGAUGAGGCCGAAAGGCCGAA ACCUUGG
2691	GUCCUCC CUGAUGAGGCCGAAAGGCCGAA AUACCUU
2700	UGGGAGG CUGAUGAGGCCGAAAGGCCGAA AGUCCUC
2704	AAGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGAGU
2711	CCUUCCA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
2712	CCCUUCC CUGAUGAGGCCGAAAGGCCGAA AAGCUGG
2721	CGCGGAU CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
2724	ACACGCG CUGAUGAGGCCGAAAGGCCGAA AUGACCC
2744	CUACACA CUGAUGAGGCCGAAAGGCCGAA ACACACA
2750	GCUUGUC CUGAUGAGGCCGAAAGGCCGAA ACACAUA
2759	AGAGCGA CUGAUGAGGCCGAAAGGCCGAA AGCUUGU
2761	ACAGAGC CUGAUGAGGCCGAAAGGCCGAA AGAGCUU
2765	GGUGACA CUGAUGAGGCCGAAAGGCCGAA AGCGAGA
2769	CCUGGGU CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
2797	GAACCAU CUGAUGAGGCCGAAAGGCCGAA AUUGCAC
2803	UGCAGUG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
2804	CUGCAGU CUGAUGAGGCCGAAAGGCCGAA AACCAUG
2813	AGGUCAA CUGAUGAGGCCGAAAGGCCGAA ACUGCAG
2815	AAAGGUC CUGAUGAGGCCGAAAGGCCGAA AGACUGC
2821	AGCCCAA CUGAUGAGGCCGAAAGGCCGAA AGGUCAA
2822	GAGCCCA CUGAUGAGGCCGAAAGGCCGAA AAGGUCA
2823	UGAGCCC CUGAUGAGGCCGAAAGGCCGAA AAAGGUC

2829	AUCACUU CUGAUGAGGCCGAAAGGCCGAA	AGCCCAA
2837	GUGGGAG CUGAUGAGGCCGAAAGGCCGAA	AUCACUU
2840	GAGGUGG CUGAUGAGGCCGAAAGGCCGAA	AGGAUCA
2847	GGAGGCU CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
2853	UACUCAG CUGAUGAGGCCGAAAGGCCGAA	AGGCUGA
2860	UCCCAGC CUGAUGAGGCCGAAAGGCCGAA	ACUCAGG
2872	GUGAGCC CUGAUGAGGCCGAAAGGCCGAA	AUGGUCC
2877	GUGUUGU CUGAUGAGGCCGAAAGGCCGAA	AGCCUAU
2899	AAAAUCA CUGAUGAGGCCGAAAGGCCGAA	AUUUGCC
2900	AAAAAUC CUGAUGAGGCCGAAAGGCCGAA	AAUUUGC
2904	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AUCAAAU
2905	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAUCAAA
2906	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAUCAA
2907	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAUCA
2908	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAUC
2909	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAU
2910	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2911	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2912	GAAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2913	UGAAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2914	CUGAAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2915	UCUGAAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2916	CUCUGAA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2917	UCUCUGA CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2918	GUCUCUG CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2919	CGUCUCU CUGAUGAGGCCGAAAGGCCGAA	AAAAAAA
2931	GUUGCGA CUGAUGAGGCCGAAAGGCCGAA	ACCCCGU
2933	AUGUUGC CUGAUGAGGCCGAAAGGCCGAA	AGACCCC
2941	UCUGGGC CUGAUGAGGCCGAAAGGCCGAA	AUGUUGC
2951	ACAAAGG CUGAUGAGGCCGAAAGGCCGAA	AGUCUGG
2952	CACAAAG CUGAUGAGGCCGAAAGGCCGAA	AAGUCUG
2955	UAACACA CUGAUGAGGCCGAAAGGCCGAA	AGGAAGU
2956	CUAACAC CUGAUGAGGCCGAAAGGCCGAA	AAGGAAG
2961	AUUAACU CUGAUGAGGCCGAAAGGCCGAA	ACACAAA
2962	UAUUAAC CUGAUGAGGCCGAAAGGCCGAA	AACACAA
2965	CUUUAUU CUGAUGAGGCCGAAAGGCCGAA	ACUAACA
2966	GCUUUAU CUGAUGAGGCCGAAAGGCCGAA	AACUAAC
2969	AAAGCUU CUGAUGAGGCCGAAAGGCCGAA	AUUAACU
2975	GUUGAGA CUGAUGAGGCCGAAAGGCCGAA	AGCUUUA
2976	AGUUGAG CUGAUGAGGCCGAAAGGCCGAA	AAGCUUU
2977	CAGUUGA CUGAUGAGGCCGAAAGGCCGAA	AAAGCUU
2979	GGCAGUU CUGAUGAGGCCGAAAGGCCGAA	AGAAAGC

Table 5

Mouse ICAM HH Ribozyme Sequence

nt. Position	Ribozyme Sequence
11	CAACGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
26	AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
31	UGUGGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CGACCCU CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
40	AGGCUAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
48	CCAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
54	CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
58	GGAGCUA CUGAUGAGGCCGAAAGGCCGAA AGGCAUG
64	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
96	GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
102	CCAGCAG CUGAUGAGGCCGAAAGGCCGAA ACUGGCA
108	GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
115	AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
119	UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCC
120	GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
146	GGAAGCG CUGAUGAGGCCGAAAGGCCGAA ACGACUG
152	AGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGA
165	GCAAAAC CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
185	CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
209	GCCAGAG CUGAUGAGGCCGAAAGGCCGAA AAGUGGC
227	GCAAAAC CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
230	GGAGCAA CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237	AGUUCUC CUGAUGAGGCCGAAAGGCCGAA AAGCACA
248	UUUAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
253	UCUUCCU CUGAUGAGGCCGAAAGGCCGAA AGGCAGG
263	CAGUAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCU
267	UAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCCCCU
293	CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
319	GGCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
335	GUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAG
337	CAGUGUG CUGAUGAGGCCGAAAGGCCGAA AUUGGAC
338	UCAGCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
359	AGCGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGCAC
367	CGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
374	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
375	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
386	AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	AGAUCGA CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
420	CGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
425	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG

427	CACUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUG
450	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
451	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
456	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUAA
495	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
510	CCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
564	GGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGAG
592	CCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
607	CAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
608	GCAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
609	GGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
611	GCGGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
656	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
657	UCAGCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
668	GGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCG
677	AGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
684	AGGACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
692	AAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
693	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
696	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
709	UGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCC
720	AGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
723	CGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUU
735	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
738	CCAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
765	GGAAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACGACUG
769	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
770	UUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA
785	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
786	AGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
792	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
794	AGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCCCAG
807	CCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCCGAG
833	GGGUGUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUG
846	CAACGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
851	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
863	CCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCU
866	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
867	UCUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AACGAAU
869	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
881	ACGGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAU
885	UCACCUC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGG
933	CCAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAG
936	GCACCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUUA
978	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
980	AAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUGU
986	AGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
987	GAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGU
988	GGAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUUG

1005	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
1006	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1023	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
1025	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCUC
1066	UUAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGUGG
1092	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
1093	UUGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1125	UCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGG
1163	GCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCG
1164	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1166	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
1172	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
1200	UGUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1201	CUGUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGC
1203	ACUGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAAAGU
1227	GCACACG	CUGAUGAGGCCGAAAGGCCGAA	AUGUACC
1228	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1233	CUCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAA
1238	AGGACCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
1264	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
1267	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1294	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
1295	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCUC
1306	CAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGC
1321	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1334	UUUAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
1344	CACUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAU
1351	UAACUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1353	CACCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACU
1366	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
1367	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
1368	AGAGUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUAC
1380	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1388	AGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
1398	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCA
1402	AGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACA
1408	CCUCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCGC
1410	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCUC
1421	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1425	CUCUACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGU
1429	CAGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
1444	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1455	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
1482	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
1484	CAUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAG
1493	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
1500	GGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAU
1503	GAAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCC
1506	CGGUUAU	CUGAUGAGGCCGAAAGGCCGAA	AACAUAA

1509	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
1518	CGCCUGG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGA
1530	CCAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAG
1533	GGCCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUGACCA
1551	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
1559	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
1563	GGUUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAG
1565	GCGGUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUA
1567	UGGCGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAAACA
1584	AUAUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
1592	UAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCU
1599	CCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGU
1651	GCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1661	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
1663	UUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUU
1678	CCAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
1680	CCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCU
1681	GCCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGC
1684	ACAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGU
1690	AGAUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGG
1691	AAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
1696	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1698	CUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCG
1737	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
1750	UGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCC
1756	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
1787	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUC
1790	AUUAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUGC
1793	UCCAGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGACCA
1797	UUUAUGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGUG
1802	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
1812	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
1813	UGAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUG
1825	GCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGUGG
1837	GGAGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
1845	GGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCG
1856	AAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
1861	UACUGGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUGU
1865	CUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUG
1868	UUUAUGU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGUG
1877	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
1901	GUCCCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUA
1912	ACUGAUC	CUGAUGAGGCCGAAAGGCCGAA	ACUAUAU
1922	UAACUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1923	GAUACCU	CUGAUGAGGCCGAAAGGCCGAA	AGCAUCA
1928	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA
1930	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
1964	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUC
1983	UAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCU

1996	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
2005	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA
2013	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2015	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
2020	CUCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCACC
2039	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2040	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2057	GGAUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
2061	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
2071	CUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUG
2076	UAGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
2097	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2098	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AUCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	CUCAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2130	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2145	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2152	AACUCUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAUA
2156	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2158	AUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUC
2159	AAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2160	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	CUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2163	AAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2166	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGU
2167	AAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2170	CUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2171	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
2173	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAACUC
2183	CAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2186	ACUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGCU
2187	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GGUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2196	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2198	AACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGC
2199	AUAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGC
2200	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
2201	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2205	UCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAA
2210	AGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
2220	AGAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
2224	GGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGAG
2226	GCGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCCA
2233	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2242	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA

2248	UGGGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUA
2254	UCAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGA
2259	CACCGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGAU
2260	GCACCGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUGA
2266	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
2274	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
2279	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
2282	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2288	AGGCCAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUAUA
2291	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACUG
2321	CCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
2338	CAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
2339	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
2341	AGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
2344	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAAA
2358	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
2359	UCUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAG
2360	UUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUU
2376	UCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACCU
2377	CUCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCACC
2378	CAGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCU
2379	CUUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCA
2380	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2382	GGGGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAU
2384	UUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAU
2399	GUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU
2401	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2411	GCAUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUA
2417	ACGUAUG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUUC
2418	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
2425	AACCCUC	CUGAUGAGGCCGAAAGGCCGAA	ACCCAUG
2426	AAACUCU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAAU
2433	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2434	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2448	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2449	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
2451	AGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
2452	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2455	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2459	GGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGG
2460	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2479	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
2480	GGAUCAC	CUGAUGAGGCCGAAAGGCCGAA	ACGGUGA
2483	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
2484	GACUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAAG
2492	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
2504	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
2508	UGGGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUA
2509	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA

2510	GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
2520	CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
2521	UGAGGGU CUGAUGAGGCCGAAAGGCCGAA AAUGCUG
2533	GAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCAUCA
2540	CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
2545	AGGACCA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
2568	UUUGACA CUGAUGAGGCCGAAAGGCCGAA ACUUCAC
2579	CAGGCCA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
2585	AGAGAAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
2588	AUUAGAG CUGAUGAGGCCGAAAGGCCGAA ACAAUGC
2591	AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
2593	GCAGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
2596	CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
2601	AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
2602	GGGAUGG CUGAUGAGGCCGAAAGGCCGAA AGCUGGA
2607	CCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCCGAG
2608	CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
2609	UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCC
2620	GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
2626	GCUGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGAAA
2628	AGGCUAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
2635	AGGACCG CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
2640	GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
2641	CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
2642	GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
2653	GCAUCCU CUGAUGAGGCCGAAAGGCCGAA ACCAGUA
2659	CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
2689	CCUCGGA CUGAUGAGGCCGAAAGGCCGAA ACAUUAG
2691	GGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGACAUU
2700	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
2704	AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
2711	CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
2712	CCCUUCC CUGAUGAGGCCGAAAGGCCGAA AGACCUC
2721	CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
2724	GCACACG CUGAUGAGGCCGAAAGGCCGAA AUGUACC
2744	CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
2750	GGUACUC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2759	AGAUCGA CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
2761	GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
2765	AGCGGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
2769	CCUGUUU CUGAUGAGGCCGAAAGGCCGAA ACAGACU
2797	GGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUUCAU
2803	CGCCUGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
2804	CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
2813	GGGUCAG CUGAUGAGGCCGAAAGGCCGAA ACCGGAG
2815	AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
2821	CCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCAG
2822	AAGUCCG CUGAUGAGGCCGAAAGGCCGAA AGGCUCC
2823	UGGGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGGCA

2829	AUGAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAG
2837	UCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACCU
2840	CAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
2847	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
2853	AACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGC
2860	UCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGC
2872	CUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCC
2877	GUGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGGAA
2899	AAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
2900	AAAACUC	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAA
2904	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGU
2905	CAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAG
2906	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2907	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2908	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
2909	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
2910	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2911	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2912	GACAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAACAA
2913	UGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAA
2914	CUUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCA
2915	UCUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
2916	CUCCGGA	CUGAUGAGGCCGAAAGGCCGAA	ACGAAUA
2917	UCUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AACGAAU
2918	CUCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAA
2919	CGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAA
2931	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
2933	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCUC
2941	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUC
2951	GCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGUGG
2952	CACAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACUGCUG
2955	UGACACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCACU
2956	UUGAUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAA
2961	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAGA
2962	AAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2965	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAA
2966	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2969	AAAACUU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUU
2975	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2976	AGUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCUC
2977	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2979	GGCAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGA

Table 6

Human ICAM Hairpin Ribozyme/Substrate Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
70	GGGCCGGG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCA GCC CCCGGCCC
86	GGAGUGCG AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCGCU GCC CGCACUCC
343	CCCAUCAG AGAA GUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAACU GCC CUGAUGGG
635	GCCCUUGG AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCG GCC CCAAGGGC
653	UGUUCUCA AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCU GUU UGAGAACA
782	AGACUGGG AGAA GCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGGCU GUU CCCAGUCU
920	CUGCACAC AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGCU GAC GUGUGCAG
1301	ACAUUGGA AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCA GAC UCCAAUGU
1373	CCCCGAUG AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACU GCC CAUCGGGG
1521	AUGACUGC AGAA GCUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAGCA GCC GCAGUCAU
1594	CUGUUGUA AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUACA GAC UACAACAG
2008	ACCCAAUA AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCU GCC UAUUGGGU
2034	UUCUGUAA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACA GAC UUACAGAA
2125	GGUCAGUA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCU GUC UACUGACC
2132	GGGUUGGG AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUACU GAC CCCAACCC
2276	ACCUGUAC AGAA GUAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUACA GUU GUACAGGU
2810	AAGGUCAA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCA GUC UUGACCUU

Table 7
Mouse ICAM Hairpin Ribozyme/Substrate Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
76	GGGAUCAC AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACC GUU GUGAUCCC
164	UGAGGAAG AGAA GUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACU GUU CUUCCUCA
252	UCAGCUCA AGAA GCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAGCU GUU UGAGCUGA
284	GCACAGCG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCA GUC CGCUGUGC
318	AAGCGGAC AGAA GCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGCA GUC GUCCGCUU
447	AGAGCUGG AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGCG GAC CCAGCUCU
804	UCUCCUGG AGAA GCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUGCC GAC CCAGGAGA
847	UCUACCAA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACU GCC UUGGUAGA
913	AGGAUCUG AGAA GCUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAGCG GAC CAGAUCCU
946	AAGUUGUA AGAA GUUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAACA GUC UACAACUU
1234	CCCAAGCA AGAA GUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGACG GAC UGCUUGGG
1275	AUUUCAGA AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCA GAC UCUGAAAU
1325	UGCCUUCC AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCA GAC GGAAGGCA
1350	CCCCGAUG AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCU GCC CAUCGGGG
1534	ACAUAAGA AGAA GCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGCA GCC UCUUAUGU
1851	GUCCACCG AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUACA GCC CGGUGGAC
1880	AGAAUGAA AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCU GAC UUCAUUCU

Table 8
Rat ICAM Hairpin Ribozyme/Substrate Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
5	AAAGUGCA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCU GCC UGCACUUU
59	GGAGCAGA AGAA GCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUGCU GCC UCUGCUCC
84	GGGAUCAC AGAA GCGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCGCC GUU GUGAUCCC
295	GCACAGUG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCA GAC CACUGUGC
329	AAGCCGAG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCA GUC CUCGGCUU
433	UUCCACCA AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCGCU GCC UGGUGGAA
626	CAUUCUUG AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACU GUU CAAGAAUG
806	UCUCCAGG AGAA GCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUGCU GAC CCUGGAGA
849	UCCACUGA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACU GCC UCAGUGGA
915	AGGGUCUG AGAA GCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGCG GAC CAGACCCU
1182	ACCUCCAA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCG GCC UUGGAGGU
1307	AUGUAAGA AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCA GAC UCUUACAU
1357	UGCUUUCC AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCA GCC GGAAAGCA
1382	UCCCGAUA AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGCU GCC UAUCGGGA
1858	GCCCACCA AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUACA GCC UGGUGGGC
1887	AGAAGGAA AGAA GCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGCU GAC UUCCUUCU
2012	GAGUUGGG AGAA GUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACACU GUC CCCAACUC
2303	AGACUCCA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACA GCC UGGAGUCU
2539	CCUCCCAC AGAA GCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAGCU GUU GUGGGAGG

Table 9: Rat ICAM HH Ribozyme Target Sequence

nt. Position	HH Target sequence	nt. Position	HH Target Sequence
11	GAUCCAAU U CACACUGA	394	GUGGUGCU U CUGAACAG
23	GCUGACUU C CUUCUCUA	420	GCACCCCU C CCAGCGCA
26	GAACUGCU C UUCCUCUU	425	CCUCGGCU U CUGCCACC
31	CCUCUGCU C CUGGUCCU	427	UCCCUGUU U AAAAACCA
34	CUGAAGCU C AGAUAUAC	450	AAGAACCU C AUCCUGCG
40	CUCAAGGU A CAAGCCCC	451	GGGUACUU C CCCCAGGC
48	GAGAACCU C GGCCUGGG	456	CUCGGCUU C UGCCACCA
54	CCCCGCCU C CCUGAGCC	495	GCCACCAU C ACUGUGUA
58	CCGUGCCU U UAGCUCCC	510	GUGCUGCU C CGUGGGAA
64	CAAUGGCU U CAACCCGU	564	GAAAAUGU U CCAACCAC
96	CCUCUGCU C CUGGUCCU	592	GGGAGUAU C ACCAGGGA
102	CUCCUGGU C CUGGUCGC	607	GAGCCAAU U UCUCAUGC
108	GGACUGCU U GGGGAACU	608	AGCCAAUU U CUCAUGCU
115	UCCUACCU U UGUUCCCA	609	GCCAAUUU C UCAUGCUU
119	GACACUGU C CCCAACUC	611	CAAUUUCU C AUGCUUCA
120	GUUGUGAU C CCCGGGCC	656	GUCACUGU U CAAGAAUG
146	CCAGACCU U GGAACUCC	657	UCACUGUU C AAGAAUGU
152	ACCCGGCU C CACCUCAA	668	GAACUGCU C UUCCUCUU
158	AUUUCUUU C ACGAGUCA	677	GCACCCCU C CCAGCGCA
165	UGAACAGU A CUUCCCCC	684	AGGCAGCU C CGGACUUU
168	GAAGCCUU C CUGCCUCG	692	CCAGACCU U GGAACUCC
185	GGGUGGAU C CGUGCAGG	693	CGGACUUU C GAUCUUCC
209	CAGCCCCU A AUCUGACC	696	GCCUGUUU C CUGCCUCU
227	GACCAAGU A ACUGUGAA	709	CAGCAUUU A CCCCUCAC
230	CAAGCUGU U GUGGGAGG	720	CUACAACU U UUCAGCUC
237	CUGAAGCU C GACACCCC	723	CAACUUUU C AGCUCCCA
248	GGCCCCCU A CCUUAGGA	735	CUCCUGGU C CUGGUCGC
253	CACUGCCU C AGUGGAGG	738	UCCUGCCU C GGGGUGGA
263	GAGCCAAU U UCUCAUGC	765	ACUGUGCU U UGAGAACU
267	GAAGCCUU C CUGCCUCG	769	UCUUGUGU U CCCUGGAA
293	GAAGCUCU U CAAGCUGA	770	CUUGUGUU C CCUGGAAG
319	CGGAGGAU C ACAAACGA	785	AGGCCUGU U UCCUGCCU
335	ACUGUGCU U UGAGAACU	786	GGCCUGUU U CCUGCCUC
337	UGUGCUAU A UGGUCCUC	792	CUCCUGGU C CUGGUCGC
338	AAGCUCUU C AAGCUGAG	794	UCCUGCCU C UGAAGCUC
359	CACGCAGU C CUCGGCUU	807	GCUCAGAU A UACCUGGA
367	CAAUGGCU U CAACCCGU	833	CCUGGGGU U GGAGACUA
374	UUACCCCU C ACCCACCU	846	CUGACAGU U AUUUAUUG
375	AGAAGCCU U CCUGCCUC	851	GCUCACCU U UAGCAGCU
378	ACCCACCU C ACAGGGUA	863	CAAUGGCU U CAACCCGU
386	CGCUGUGU U UUGGAGCU	866	CCAUGCUU C CUCUGACA

867	GACCACCU C CCCACCUA	1421	GGGUACUU C CCCCAGGC
869	CUCUUCCU C UUGCGAAG	1425	ACCCACCU C CUCUGGCU
881	AAUGGCUU C AACCCGUG	1429	AUACUUGU A GCCUCAGG
885	GACCAAGU A ACUGUGAA	1444	AGAAGGCU C AGGAGGAG
933	UGUGUAUU C GUUCCCAG	1455	GGGAGUAU C ACCAGGGA
936	GCAGAGAU U UUGUGUCA	1482	AGGGUACU U CCCCCAGG
978	UUGAGAAU C UACAACUU	1484	ACUGCUCU U CCUCUUGC
980	GAGAAUCU A CAACUUUU	1493	CCUGGGGU U GGAGACUA
986	CUACAACU U UUCAGCUC	1500	CGUGAAAU U AUGGUCAA
987	UACAACUU U UCAGCUCC	1503	GAAAAUGU U CCAACCAC
988	ACAACUUU U CAGCUCCC	1506	UGGGUCAU A AUUGUUGG
1005	UUCGUGAU C GUGGCGUC	1509	GCCACCAU C ACUGUGUA
1006	GUGGGAGU A UCACCAGG	1518	GUCCUGGU C GCCGUUGU
1023	CCGGAGGU C UCAGAAGG	1530	ACCUGGGU C AUAAUUGU
1025	GGAGGUCU C AGAAGGGG	1533	CUGAUCAU U GCGGGCUU
1066	CCUACCUU U GUUCCCAA	1551	GUGGCCCU C UGCUCGUA
1092	AGAGGGGU C UCAGCAGA	1559	UGGGAAGU C CCUGUUUA
1093	AGGGGAAU C CAGCCCCU	1563	UCCUACCU U UGUUCCCA
1125	CCCCAACU C UUGUUGAU	1565	UUACACCU A UUACCGCC
1163	ACGACGCU U CUUUUGCU	1567	ACACCUAU U ACCGCCAG
1164	CGACGCUU C UUUUGCUC	1584	AGGAAGAU C AGGAUAUA
1166	ACGCUUCU U UUGCUCUG	1592	CAGGAUAU A CAAGUUAC
1172	CUUUUGCU C UGCGGCCU	1599	UACAAGUU A CAGAAGGC
1200	AUCCAAUU C ACACUGAA	1651	CCCCGCCU C CCUGAGCC
1201	UUGGGCUU C UCCACAGG	1661	CUGCACUU U GCCCUGGU
1203	GGGCUUCU C CACAGGUC	1663	GAACAGAU C AAUGGACA
1227	UUGGAACU C CAUGUGCU	1678	GAGAACCU C GGCCUGGG
1228	GCGGGCUU C GUGAUCGU	1680	GGGCUUCU C CACAGGUC
1233	CUCCUGGU C CUGGUCGC	1681	GGCCUGUU U CCUGCCUC
1238	UGUGCUAU A UGGUCCUC	1684	CUGCUCGU A GACCUCUC
1264	GGAAAGAU C AUACGGGU	1690	CCCCACCU A CAUACAUU
1267	GUCACUGU U CAAGAAUG	1691	CCGGACUU U CGAUCUUC
1294	CAGAGAUU U UGUGUCAG	1696	CUCCUGGU C CUGGUCGC
1295	AGAGGGGU C UCAGCAGA	1698	UCAGAUAU A CCUGGAGA
1306	AGCAGACU C UUACAUGC	1737	GAUCACAU U CACGGUGC
1321	AACAGAGU C UGGGGAAA	1750	GUCCAUUU A CACCUAUU
1334	GUAUUCGU U CCCAGAGC	1756	CCUCUGCU C CUGGUCCU
1344	UCGGUGCU C AGGUAUCC	1787	GAGAACCU C GGCCUGGG
1351	UCAGGCCU A AGAGGACU	1790	GACACUGU C CCCAACUC
1353	UAGCAGCU C AACAAUGG	1793	AUGGUCCU C ACCUGGAC
1366	AGGGUACU U CCCCCAGG	1797	UCCCUGUU U AAAAACCA
1367	GGGUACUU C CCCCAGGC	1802	GCUCAGAU A UACCUGGA
1368	GAUGGUGU C CCGCUGCC	1812	AACAGAGU C UGGGGAAA
1380	CUGCCUAU C GGGAUGGU	1813	GCGGGCUU C GUGAUCGU
1388	UGGAGACU A ACUGGAUG	1825	GCCACCAU C ACUGUGUA
1398	CUGGCUGU C ACAGGACA	1837	ACCCACCU C ACAGGGUA
1402	CUGUGCUU U GAGAACUG	1845	AGAGGACU C GGAGGGGC
1408	UUCGUGAU C GUGGCGUC	1856	CCCCUAAU C UGACCUGC
1410	CGAACUAU C GAGUGGAC	1861	CAUGUGCU A UAUGGUCC

1865	UAUCCGGU A GACACAAG	2198	GAAUGUCU C CGAGGUCA
1868	UCACGAGU C AUAUAAAU	2199	AGACUCUU A CAUGCCAG
1877	ACAGUACU U CCCCCAGG	2200	GGGUACUU C CCCCAGGC
1901	CUAAAACU C AAGGUACA	2201	GGGCUUCU C CACAGGUC
1912	GAACAGAU C AAUGGACA	2205	UUUUGUGU C AGCCACUG
1922	AUGUAAGU U AUUGCCUA	2210	UGGAGACU A ACUGGAUG
1923	UGGACGCU C ACCUUUAG	2220	GAGAACCU C GGCCUGGG
1928	GCUCAGAU A UACCUGGA	2224	ACAUACAU U CCUACCUU
1930	UGGAGACU A ACUGGAUG	2226	CUGGACCU C AGGCCACA
1964	AGAGAUUU U GUGUCAGC	2233	UCAUGCUU C ACAGAACU
1983	GAGAACCU C GGCCUGGG	2242	ACACAGCU C UCAGUAGU
1996	UGGAAGCU C UUCAAGCU	2248	CUCCUGGU C CUGGUCGC
2005	AUGUAAGU U AUUGCCUA	2254	AUCCAAUU C ACACUGAA
2013	CGCUGCCU A UCGGGAUG	2259	GAUCACAU U CACGGUGC
2015	CUGCCUAU C GGGAUGGU	2260	AUCACAUU C ACGGUGCU
2020	UAUUGAGU A CCCUGUAC	2266	AUCAGGAU A UACAAGUU
2039	CGGAGGAU C ACAAACGA	2274	GAGCAGGU U AACAUGUA
2040	CCUGACCU C CUGGAGGU	2279	GGAAAGAU C AUACGGGU
2057	CUGGUCCU C CAAUGGCU	2282	ACAGUUAU U UAUUGAGU
2061	GCGUCCAU U UACACCUA	2288	GCCCUGGU C CUCCAAUG
2071	AUACUUGU A GCCUCAGG	2291	CAGGAUAU A CAAGUUAC
2076	UGUAGCCU C AGGCCUAA	2321	GGAAAGAU C AUACGGGU
2097	CCAACUCU U GUUGAUGU	2338	UUGGGCUU C UCCACAGG
2098	CCUGACCU C CUGGAGGU	2339	GGGUACUU C CCCCAGGC
2115	UUCCGACU A GGGUCCUG	2341	GGGCCUGU C GGUGCUCA
2128	AGUGCUGU A CCAUGAUC	2344	CUGCUCGU A GACCUCUC
2130	GCCUGUUU C CUGCCUCU	2358	CCCUGCCU C CUCCCACA
2145	CCAACUCU U GUUGAUGU	2359	CCAUCCAU C CCACAGAA
2152	UUGAGAAU C UACAACUU	2360	CUUGUGUU C CCUGGAAG
2156	UGACAGUU A UUUAUUGA	2376	GAACUGCU C UUCCUCUU
2158	UGAUGUAU U UAUUAAUU	2377	GACUUCCU U CUCUAUUA
2159	GAUGUAUU U AUUAAUUC	2378	GCUGAUUU C UUUCACGA
2160	AUGUAUUU A UUAAUUCA	2379	CUGCUCUU C CUCUUGCG
2162	ACAUUCCU A CCUUUGUU	2380	UGAUUUCU U UCACGAGU
2163	UAUUUAUU A AUUCAGAG	2382	AUUUCUUU C ACGAGUCA
2166	UGAUGUAU U UAUUAAUU	2384	UAUCCGGU A GACACAAG
2167	GAUGUAUU U AUUAAUUC	2399	UAAAUACU A UGUGGACG
2170	GUAUUUAU U AAUUCAGA	2401	UGUGCUAU A UGGUCCUC
2171	CAGUUAUU U AUUGAGUA	2411	CAAUUUCU C AUGCUUCA
2173	UGUGCUAU A UGGUCCUC	2417	AUCAGGAU A UACAAGUU
2174	UCUCUAUU A CCCCUGCU	2418	UCAUGCUU C ACAGAACU
2175	AUUUCUUU C ACGAGUCA	2425	UUAUUAAU U CAGAGUUC
2176	GAAAAUGU U CCAACCAC	2426	CCUGGGGU U GGAGACUA
2183	UGACAGUU A UUUAUUGA	2433	UCAGAGUU C UGACAGUU
2185	ACAGUUAU U UAUUGAGU	2434	CGGAGGAU C ACAAACGA
2186	CAGUUAUU U AUUGAGUA	2448	UGAACAGU A CUUCCCCC
2187	AGUUAUUU A UUGAGUAC	2449	GAAGCCUU C CUGCCUCG
2189	UUAUUUAU U GAGUACCC	2451	GGCCUGUU U CCUGCCUC
2196	CUGACAGU U AUUUAUUG	2452	GCCUGUUU C CUGCCUCU

2455	ACAUUCCU A CCUUUGUU	2761	CGGACUUU C GAUCUUCC
2459	CCCUGCCU C CUCCCACA	2765	CUUUUGCU C UGCGGCCU
2460	CCUACCUU U GUUCCCAA	2769	UUCUCUAU U ACCCCUGC
2479	UUACACCU A UUACCGCC	2797	CGUGAAAU U AUGGUCAA
2480	GUCGCCGU U GUGAUCCC	2803	CUCAUGCU U CACAGAAC
2483	ACCUUUGU U CCCAAUGU	2804	UCAUGCUU C ACAGAACU
2484	CCUUUGUU C CCAAUGUC	2813	GCUCCCAU C CUGACCCU
2492	GACCACCU C CCCACCUA	2815	CGGACUUU C GAUCUUCC
2504	ACCUACAU A CAUUCCUA	2821	CCUGACCU C CUGGAGGU
2508	ACAUACAU U CCUACCUU	2822	UACAACUU U UCAGCUCC
2509	CAUACAUU C CUACCUUU	2823	CAACUUUU C AGCUCCCA
2510	GUCCAUUU A CACCUAUU	2829	UCGGUGCU C AGGUAUCC
2520	ACCUUUGU U CCCAAUGU	2837	CACAGGGU A CUUCCCCC
2521	CCUUUGUU C CCAAUGUC	2840	GCACCCCU C CCAGCGCA
2533	ACAGCAUU U ACCCCUCA	2847	UUACCCCU C ACCCACCU
2540	UCGGUGCU C AGGUAUCC	2853	UUCGAUCU U CCGACUAG
2545	AGGCAGCU C CGGACUUU	2860	UCUUGUGU U CCCUGGAA
2568	CAGAGAUU U UGUGUCAG	2872	GGGCCUGU C GGUGCUCA
2579	CCUGCACU U UGCCCUGG	2877	UGGAGUCU C CCAGCACC
2585	CUGCUCGU A GACCUCUC	2899	AGGCAGCU C CGGACUUU
2588	UGCCUCCU C CCACAGCC	2900	GGCUGACU U CCUUCUCU
2591	CUCUUCCU C UUGCGAAG	2904	GAACUGCU C UUCCUCUU
2593	UCUCUAUU A CCCCUGCU	2905	GGCUGACU U CCUUCUCU
2596	CUCCUGGU C CUGGUCGC	2906	GUUGAUGU A UUUAUUAA
2601	UGUGCUAU A UGGUCCUC	2907	CUGCUCUU C CUCUUGCG
2602	GUCCUGGU C GCCGUUGU	2908	UGAUGUAU U UAUUAAUU
2607	GUGGGAGU A UCACCAGG	2909	GAACUGCU C UUCCUCUU
2608	CUUUAGCU C CCGUGGGA	2910	ACUUCCUU C UCUAUUAC
2609	UGGAGACU A ACUGGAUG	2911	UUCCUUCU C UAUUACCC
2620	UCAGAGUU C UGACAGUU	2912	AUGUAUUU A UUAAUUCA
2626	CUCUCAGU A GUGCUGCU	2913	UGUGUAUU C GUUCCCAG
2628	UACAACUU U UCAGCUCC	2914	GUAUUUAU U AAUUCAGA
2635	UCACAGAU C CAAUUCAC	2915	UAUUUAUU A AUUCAGAG
2640	GCUCAGGU A UCCAUCCA	2916	CUCUUCCU C UUGCGAAG
2641	CCCCACCU A CAUACAUU	2917	CUUCCUCU U GCGAAGAC
2642	GCCUGUUU C CUGCCUCU	2918	AUUUCUUU C ACGAGUCA
2653	CCACAGGU C AGGGUGCU	2919	UUUUGUGU C AGCCACUG
2659	AGAAGGGU C CUGCAAGC	2931	GAUGGUGU C CCGCUGCC
2689	ACUAGGGU C CUGAAGCU	2933	UGGAGUCU C CCAGCACC
2691	UCAGGCCU A AGAGGACU	2941	CAGUACUU C CCCCAGGC
2700	AGGGUACU U CCCCCAGG	2951	ACCAUGCU U CCUCUGAC
2704	GACCACCU C CCCACCUA	2952	CCGGACUU U CGAUCUUC
2711	CCCUACCU U AGGAAGGU	2955	UGCUUCCU C UGACAUGG
2712	CCUACCUU A GGAAGGUG	2956	CUUUCCUU U GAAUCAAU
2721	GGAAAGAU C AUACGGGU	2961	UUUUGUGU C AGCCACUG
2724	AAGAUCAU A CGGGUUUG	2962	UGUGUAUU C GUUCCCAG
2744	GGGUGGAU C CGUGCAGG	2965	CUUUGAAU C AAUAAAGU
2750	GUCCCUGU U UAAAAACC	2966	UGGAAGCU C UUCAAGCU
2759	GACGAACU A UCGAGUGG	2969	GAAUCAAU A AAGUUUUA

2975	UGGAAGCU C UUCAAGCU
2976	UAUAUGGU C CUCACCUG
2977	GAAGCUCU U CAAGCUGA

Table 10: Rat ICAM HH Ribozyme Sequences

nt. Position	Rat HH Ribozyme Sequence
11	UCAGUGUG CUGAUGAGGCCGAAAGGCCGAA AUUGGAUC
23	UAGAGAAG CUGAUGAGGCCGAAAGGCCGAA AAGUCAGC
26	AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCAGUUC
31	AGGACCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAGG
34	GUAUAUCU CUGAUGAGGCCGAAAGGCCGAA AGCUUCAG
40	GGGGCUUG CUGAUGAGGCCGAAAGGCCGAA ACCUUGAG
48	CCCAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCUC
54	GGCUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCGGGG
58	GGGAGCUA CUGAUGAGGCCGAAAGGCCGAA AGGCACGG
64	ACGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUUG
96	AGGACCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAGG
102	GCGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
108	AGUUCCCC CUGAUGAGGCCGAAAGGCCGAA AGCAGUCC
115	UGGGAACA CUGAUGAGGCCGAAAGGCCGAA AGGUAGGA
119	GAGUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGUGUC
120	GGCCCGGG CUGAUGAGGCCGAAAGGCCGAA AUCACAAC
146	GGAGUUCC CUGAUGAGGCCGAAAGGCCGAA AGGUCUGG
152	UUGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGGGU
158	UGACUCGU CUGAUGAGGCCGAAAGGCCGAA AAAGAAAU
165	GGGGGAAG CUGAUGAGGCCGAAAGGCCGAA ACUGUUCA
168	CGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUUC
185	CCUGCACG CUGAUGAGGCCGAAAGGCCGAA AUCCACCC
209	GGUCAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGCUG
227	UUCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUUGGUC
230	CCUCCCAC CUGAUGAGGCCGAAAGGCCGAA ACAGCUUG
237	GGGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGCUUCAG
248	UCCUAAGG CUGAUGAGGCCGAAAGGCCGAA AGGGGGCC
253	CCUCCACU CUGAUGAGGCCGAAAGGCCGAA AGGCAGUG
263	GCAUGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCUC
267	CGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUUC
293	UCAGCUUG CUGAUGAGGCCGAAAGGCCGAA AGAGCUUC
319	UCGUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCCUCCG
335	AGUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAGU
337	GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
338	CUCAGCUU CUGAUGAGGCCGAAAGGCCGAA AAGAGCUU
359	AAGCCGAG CUGAUGAGGCCGAAAGGCCGAA ACUGCGUG
367	ACGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUUG
374	AGGUGGGU CUGAUGAGGCCGAAAGGCCGAA AGGGGUAA
375	GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUCU
378	UACCCUGU CUGAUGAGGCCGAAAGGCCGAA AGGUGGGU
386	AGCUCCAA CUGAUGAGGCCGAAAGGCCGAA ACACAGCG

394	CUGUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACCAC
420	UGCGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUGC
425	GGUGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGAGG
427	UGGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGGA
450	CGCAGGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUU
451	GCCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACCC
456	UGGUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCGAG
495	UACACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGGC
510	UUCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCAC
564	GUGGUUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUUC
592	UCCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUACUCCC
607	GCAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCUC
608	AGCAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGCU
609	AAGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGGC
611	UGAAGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUUG
656	CAUUCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUGAC
657	ACAUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGUGA
668	AAGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUUC
677	UGCGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUGC
684	AAAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCCU
692	GGAGUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUGG
693	GGAAGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCCG
696	AGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGGC
709	GUGAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGCUG
720	GAGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUAG
723	UGGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUUG
735	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
738	UCCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGA
765	AGUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAGU
769	UUCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAGA
770	CUUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACACAAG
785	AGGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCCU
786	GAGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGCC
792	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
794	GAGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGA
807	UCCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAGC
833	UAGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCCCAGG
846	CAAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCAG
851	AGCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAGC
863	ACGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUUG
866	UGUCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGG
867	UAGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGUC
869	CUUCGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGAG
881	CACGGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAUU
885	UUCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGUC
933	CUGGGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUACACA
936	UGACACAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUGC
978	AAGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCAA
980	AAAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCUC

986	GAGCUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUAG
987	GGAGCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUUGUA
988	GGGAGCUG CUGAUGAGGCCGAAAGGCCGAA AAAGUUGU
1005	GACGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCACGAA
1006	CCUGGUGA CUGAUGAGGCCGAAAGGCCGAA ACUCCCAC
1023	CCUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCGG
1025	CCCCUUCU CUGAUGAGGCCGAAAGGCCGAA AGACCUCC
1066	UUGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
1092	UCUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUCU
1093	AGGGGCUG CUGAUGAGGCCGAAAGGCCGAA AUUCCCCU
1125	AUCAACAA CUGAUGAGGCCGAAAGGCCGAA AGUUGGGG
1163	AGCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGCGUCGU
1164	GAGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGCGUCG
1166	CAGAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGCGU
1172	AGGCCGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAAG
1200	UUCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGAU
1201	CCUGUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCCCAA
1203	GACCUGUG CUGAUGAGGCCGAAAGGCCGAA AGAAGCCC
1227	AGCACAUG CUGAUGAGGCCGAAAGGCCGAA AGUUCCAA
1228	ACGAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGCCCGC
1233	GCGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
1238	GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
1264	ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA AUCUUUCC
1267	CAUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACAGUGAC
1294	CUGACACA CUGAUGAGGCCGAAAGGCCGAA AAUCUCUG
1295	UCUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUCU
1306	GCAUGUAA CUGAUGAGGCCGAAAGGCCGAA AGUCUGCU
1321	UUUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUGUU
1334	GCUCUGGG CUGAUGAGGCCGAAAGGCCGAA ACGAAUAC
1344	GGAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCACCGA
1351	AGUCCUCU CUGAUGAGGCCGAAAGGCCGAA AGGCCUGA
1353	CCAUUGUU CUGAUGAGGCCGAAAGGCCGAA AGCUGCUA
1366	CCUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
1367	GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
1368	GGCAGCGG CUGAUGAGGCCGAAAGGCCGAA ACACCAUC
1380	ACCAUCCC CUGAUGAGGCCGAAAGGCCGAA AUAGGCAG
1388	CAUCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUCUCCA
1398	UGUCCUGU CUGAUGAGGCCGAAAGGCCGAA ACAGCCAG
1402	CAGUUCUC CUGAUGAGGCCGAAAGGCCGAA AAGCACAG
1408	GACGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCACGAA
1410	GUCCACUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUCG
1421	GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
1425	AGCCAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUGGGU
1429	CCUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAAGUAU
1444	CUCCUCCU CUGAUGAGGCCGAAAGGCCGAA AGCCUUCU
1455	UCCCUGGU CUGAUGAGGCCGAAAGGCCGAA AUACUCCC
1482	CCUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
1484	GCAAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGCAGU
1493	UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG

1500	UUGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCACG
1503	GUGGUUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUUC
1506	CCAACAAU	CUGAUGAGGCCGAAAGGCCGAA	AUGACCCA
1509	UACACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGGC
1518	ACAACGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAC
1530	ACAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGU
1533	AAGCCCGC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCAG
1551	UACGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCAC
1559	UAAACAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUCCCA
1563	UGGGAACA	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGGA
1565	GGCGGUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUAA
1567	CUGGCGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGGUGU
1584	UAUAUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCCU
1592	GUAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCUG
1599	GCCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGUA
1651	GGCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGGGG
1661	ACCAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGCAG
1663	UGUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUUC
1678	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
1680	GACCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCCC
1681	GAGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGCC
1684	GAGAGGUC	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCAG
1690	AAUGUAUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGG
1691	GAAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCGG
1696	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
1698	UCUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCUGA
1737	GCACCGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGAUC
1750	AAUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGGAC
1756	AGGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAGG
1787	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
1790	GAGUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUGUC
1793	GUCCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGACCAU
1797	UGGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGGA
1802	UCCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAGC
1812	UUUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGUU
1813	ACGAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCGC
1825	UACACAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGGC
1837	UACCCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGU
1845	GCCCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUCU
1856	GCAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUAGGGG
1861	GGACCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAUG
1865	CUUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACCGGAUA
1868	AUUUAUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUCGUGA
1877	CCUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUACUGU
1901	UGUACCUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUAG
1912	UGUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUUC
1922	UAGGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUACAU
1923	CUAAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGCGUCCA
1928	UCCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAGC

1930	CAUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCCA
1964	GCUGACAC	CUGAUGAGGCCGAAAGGCCGAA	AAAUCUCU
1983	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
1996	AGCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCCA
2005	UAGGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUACAU
2013	CAUCCCGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGCG
2015	ACCAUCCC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGCAG
2020	GUACAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACUCAAUA
2039	UCGUUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCCG
2040	ACCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAGG
2057	AGCCAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGACCAG
2061	UAGGUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGACGC
2071	CCUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUAU
2076	UUAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUACA
2097	ACAUCAAC	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUGG
2098	ACCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAGG
2115	CAGGACCC	CUGAUGAGGCCGAAAGGCCGAA	AGUCGGAA
2128	GAUCAUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCACU
2130	AGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGGC
2145	ACAUCAAC	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUGG
2152	AAGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCAA
2156	UCAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AACUGUCA
2158	AAUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUCA
2159	GAAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAUC
2160	UGAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACAU
2162	AACAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUGU
2163	CUCUGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAUA
2166	AAUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUCA
2167	GAAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAUC
2170	UCUGAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUAC
2171	UACUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAACUG
2173	GAGGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCACA
2174	AGCAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAGA
2175	UGACUCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAAU
2176	GUGGUUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUUC
2183	UCAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AACUGUCA
2185	ACUCAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAACUGU
2186	UACUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAACUG
2187	GUACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAACU
2189	GGGUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUAA
2196	CAAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCAG
2198	UGACCUCG	CUGAUGAGGCCGAAAGGCCGAA	AGACAUUC
2199	CUGGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUCU
2200	GCCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACCC
2201	GACCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCCC
2205	CAGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAAAA
2210	CAUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCCA
2220	CCCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCUC
2224	AAGGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUGU

2226	UGUGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCAG
2233	AGUUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGCAUGA
2242	ACUACUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGUGU
2248	GCGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
2254	UUCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGAU
2259	GCACCGUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAUC
2260	AGCACCGU CUGAUGAGGCCGAAAGGCCGAA AAUGUGAU
2266	AACUUGUA CUGAUGAGGCCGAAAGGCCGAA AUCCUGAU
2274	UACAUGUU CUGAUGAGGCCGAAAGGCCGAA ACCUGCUC
2279	ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA AUCUUUCC
2282	ACUCAAUA CUGAUGAGGCCGAAAGGCCGAA AUAACUGU
2288	CAUUGGAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGGC
2291	GUAACUUG CUGAUGAGGCCGAAAGGCCGAA AUAUCCUG
2321	ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA AUCUUUCC
2338	CCUGUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCCCAA
2339	GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
2341	UGAGCACC CUGAUGAGGCCGAAAGGCCGAA ACAGGCCC
2344	GAGAGGUC CUGAUGAGGCCGAAAGGCCGAA ACGAGCAG
2358	UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
2359	UUCUGUGG CUGAUGAGGCCGAAAGGCCGAA AUGGAUGG
2360	CUUCCAGG CUGAUGAGGCCGAAAGGCCGAA AACACAAG
2376	AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCAGUUC
2377	UAAUAGAG CUGAUGAGGCCGAAAGGCCGAA AGGAAGUC
2378	UCGUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCAGC
2379	CGCAAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGCAG
2380	ACUCGUGA CUGAUGAGGCCGAAAGGCCGAA AGAAAUCA
2382	UGACUCGU CUGAUGAGGCCGAAAGGCCGAA AAAGAAAU
2384	CUUGUGUC CUGAUGAGGCCGAAAGGCCGAA ACCGGAUA
2399	CGUCCACA CUGAUGAGGCCGAAAGGCCGAA AGUAUUUA
2401	GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
2411	UGAAGCAU CUGAUGAGGCCGAAAGGCCGAA AGAAAUUG
2417	AACUUGUA CUGAUGAGGCCGAAAGGCCGAA AUCCUGAU
2418	AGUUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGCAUGA
2425	GAACUCUG CUGAUGAGGCCGAAAGGCCGAA AUUAAUAA
2426	UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG
2433	AACUGUCA CUGAUGAGGCCGAAAGGCCGAA AACUCUGA
2434	UCGUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCCUCCG
2448	GGGGGAAG CUGAUGAGGCCGAAAGGCCGAA ACUGUUCA
2449	CGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUUC
2451	GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGCC
2452	AGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGGC
2455	AACAAAGG CUGAUGAGGCCGAAAGGCCGAA AGGAAUGU
2459	UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
2460	UUGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
2479	GGCGGUAA CUGAUGAGGCCGAAAGGCCGAA AGGUGUAA
2480	GGGAUCAC CUGAUGAGGCCGAAAGGCCGAA ACGGCGAC
2483	ACAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAGGU
2484	GACAUUGG CUGAUGAGGCCGAAAGGCCGAA AACAAAGG
2492	UAGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGUC

2504	UAGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUAGGU
2508	AAGGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUGU
2509	AAAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGUAUG
2510	AAUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGGAC
2520	ACAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAGGU
2521	GACAUUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAAAGG
2533	UGAGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUGU
2540	GGAUACCU	CUGAUGAGGCCGAAAGGCCGAA	AGCACCGA
2545	AAAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCCU
2568	CUGACACA	CUGAUGAGGCCGAAAGGCCGAA	AAUCUCUG
2579	CCAGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAGG
2585	GAGAGGUC	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCAG
2588	GGCUGUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGCA
2591	CUUCGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGAG
2593	AGCAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAGA
2596	GCGACCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAG
2601	GAGGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCACA
2602	ACAACGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGAC
2607	CCUGGUGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCCAC
2608	UCCCACGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUAAAG
2609	CAUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCCA
2620	AACUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUGA
2626	AGCAGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGAG
2628	GGAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGUA
2635	GUGAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUGA
2640	UGGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGAGC
2641	AAUGUAUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGGG
2642	AGAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGGC
2653	AGCACCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUGG
2659	GCUUGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUCU
2689	AGCUUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUAGU
2691	AGUCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUGA
2700	CCUGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUACCCU
2704	UAGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGUC
2711	ACCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGGG
2712	CACCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAGG
2721	ACCCGUAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUCC
2724	CAAACCCG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCUU
2744	CCUGCACG	CUGAUGAGGCCGAAAGGCCGAA	AUCCACCC
2750	GGUUUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGAC
2759	CCACUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGUUCGUC
2761	GGAAGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCCG
2765	AGGCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAAG
2769	GCAGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGAA
2797	UUGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCACG
2803	GUUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUGAG
2804	AGUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGA
2813	AGGGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAGC
2815	GGAAGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCCG

2821	ACCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAGG
2822	GGAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGUA
2823	UGGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUUG
2829	GGAUACCU	CUGAUGAGGCCGAAAGGCCGAA	AGCACCGA
2837	GGGGGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCUGUG
2840	UGCGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUGC
2847	AGGUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUAA
2853	CUAGUCGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCGAA
2860	UUCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAGA
2872	UGAGCACC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCCC
2877	GGUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGACUCCA
2899	AAAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCCU
2900	AGAGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGCC
2904	AAGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUUC
2905	AGAGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGCC
2906	UUAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAAC
2907	CGCAAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGCAG
2908	AAUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUCA
2909	AAGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUUC
2910	GUAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAGU
2911	GGGUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGGAA
2912	UGAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACAU
2913	CUGGGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUACACA
2914	UCUGAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUAC
2915	CUCUGAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAUA
2916	CUUCGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGAG
2917	GUCUUCGC	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAAG
2918	UGACUCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAAU
2919	CAGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAAAA
2931	GGCAGCGG	CUGAUGAGGCCGAAAGGCCGAA	ACACCAUC
2933	GGUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGACUCCA
2941	GCCUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUACUG
2951	GUCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUGGU
2952	GAAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCGG
2955	CCAUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGCA
2956	AUUGAUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAAG
2961	CAGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAAAA
2962	CUGGGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUACACA
2965	ACUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAAG
2966	AGCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCCA
2969	UAAAACUU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUUC
2975	AGCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCCA
2976	CAGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUAUA
2977	UCAGCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUUC

Table 11: Human IL-5 HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AUGCACU U UCUUUGC	245	AAGAAAU C UUUCAGG
9	UGCACUU U CUUUGCC	247	GAAAUCU U UCAGGGA
10	GCACUUU C UUUGCCA	248	AAAUCUU U CAGGGAA
12	ACUUUCU U UGCCAAA	249	AAUCUUU C AGGGAAU
13	CUUUCUU U GCCAAAG	257	AGGGAAU A GGCACAC
36	AGAACGU U UCAGAGC	273	GGAGAGU C AAACUGU
37	GAACGUU U CAGAGCC	291	AGGGGGU A CUGUGGA
38	AACGUUU C AGAGCCA	305	AAAGACU A UUCAAAA
56	GGAUGCU U CUGCAUU	307	AGACUAU U CAAAAAC
57	GAUGCUU C UGCAUUU	308	GACUAUU C AAAAACU
63	UCUGCAU U UGAGUUU	316	AAAAACU U GUCCUUA
64	CUGCAUU U GAGUUUG	319	AACUUGU C CUUAAUA
69	UUUGAGU U UGCUAGC	322	UUGUCCU U AAUAAAG
70	UUGAGUU U GCUAGCU	323	UGUCCUU A AUAAAGA
74	GUUUGCU A GCUCUUG	326	CCUUAAU A AAGAAAU
78	GCUAGCU C UUGGAGC	334	AAGAAAU A CAUUGAC
80	UAGCUCU U GGAGCUG	338	AAUACAU U GACGGCC
91	GCUGCCU A CGUGUAU	380	GGAGAGU A AACCAAU
97	UACGUGU A UGCCAUC	388	AACCAAU U CCUAGAC
104	AUGCCAU C CCCACAG	389	ACCAAUU C CUAGACU
116	CAGAAAU U CCCACAA	392	AAUUCCU A GACUACC
117	AGAAAUU C CCACAAG	397	CUAGACU A CCUGCAA
130	AGUGCAU U GGUGAAA	409	CAAGAGU U UCUUGGU
145	GAGACCU U GGCACUG	410	AAGAGUU U CUUGGUG
155	CACUGCU U UCUACUC	411	AGAGUUU C UUGGUGU
156	ACUGCUU U CUACUCA	413	AGUUUCU U GGUGUAA
157	CUGCUUU C UACUCAU	419	UUGGUGU A AUGAACA
159	GCUUUCU A CUCAUCG	437	AGUGGAU A AUAGAAA
162	UUCUACU C AUCGAAC	440	GGAUAAU A GAAAGUU
165	UACUCAU C GAACUCU	447	AGAAAGU U GAGACUA
171	UCGAACU C UGCUGAU	454	UGAGACU A AACUGGU
179	UGCUGAU A GCCAAUG	462	AACUGGU U UGUUGCA
192	UGAGACU C UGAGGAU	463	ACUGGUU U GUUGCAG
200	UGAGGAU U CCUGUUC	466	GGUUUGU U GCAGCCA
201	GAGGAUU C CUGUUCC	479	CAAAGAU U UUGGAGG
206	UUCCUGU U CCUGUAC	480	AAAGAUU U UGGAGGA
207	UCCUGUU C CUGUACA	481	AAGAUUU U GGAGGAG
212	UUCCUGU A CAUAAAA	497	AGGACAU U UUACUGC
216	UGUACAU A AAAAUCA	498	GGACAUU U UACUGCA
222	UAAAAAU C ACCAACU	499	GACAUUU U ACUGCAG

500	ACAUUUU A CUGCAGU
531	AAAGAGU C AGGCCUU
538	CAGGCCU U AAUUUUC
539	AGGCCUU A AUUUUCA
542	CCUUAAU U UUCAAUA
543	CUUAAUU U UCAAUAU
544	UUAAUUU U CAAUAUA
545	UAAUUUU C AAUAUAA
549	UUUCAAU A UAAUUUA
551	UCAAUAU A AUUUAAC
554	AUAUAAU U UAACUUC
555	UAUAAUU U AACUUCA
556	AUAAUUU A ACUUCAG
560	UUUAACU U CAGAGGG
561	UUAACUU C AGAGGGA
573	GGAAAGU A AAUAUUU
577	AGUAAAU A UUUCAGG
579	UAAAUAU U UCAGGCA
580	AAAUAUU U CAGGCAU
581	AAUAUUU C AGGCAUA
588	CAGGCAU A CUGACAC
597	UGACACU U UGCCAGA
598	GACACUU U GCCAGAA
611	AAAGCAU A AAAUUCU
616	AUAAAAU U CUUAAAA
617	UAAAAUU C UUAAAAU
619	AAAUUCU U AAAAUAU
620	AAUUCUU A AAAUAUA
625	UUAAAAU A UAUUUCA
627	AAAAUAU A UUUCAGA
629	AAUAUAU U UCAGAUA
630	AUAUAUU U CAGAUAU
631	UAUAUUU C AGAUAUC
636	UUCAGAU A UCAGAAU
638	CAGAUAU C AGAAUCA
644	UCAGAAU C AUUGAAG
647	GAAUCAU U GAAGUAU
653	UUGAAGU A UUUUCCU
655	GAAGUAU U UUCCUCC
656	AAGUAUU U UCCUCCA
657	AGUAUUU U CCUCCAG
658	GUAUUUU C CUCCAGG
661	UUUUCCU C CAGGCAA
672	GCAAAAU U GAUAUAC
676	AAUUGAU A UACUUUU
578	UUGAUAU A CUUUUUU
581	AUAUACU U UUUUCUU
682	UAUACUU U UUUCUUA
684	UACUUUU U UCUUAUU
685	ACUUUUU U CUUAUUU
686	CUUUUUU C UUAUUUA
688	UUUUUCU U AUUUAAC
689	UUUUCUU A UUUAACU
691	UUCUUAU U UAACUUA
692	UCUUAUU U AACUUAA
693	CUUAUUU A ACUUAAC
697	UUUAACU U AACAUUC
698	UUAACUU A ACAUUCU
703	UUAACAU U CUGUAAA
704	UAACAUU C UGUAAAA
708	AUUCUGU A AAAUGUC
715	AAAAUGU C UGUUAAC
719	UGUCUGU U AACUUAA
720	GUCUGUU A ACUUAAU
724	GUUAACU U AAUAGUA
725	UUAACUU A AUAGUAU
728	ACUUAAU A GUAUUUA
731	UAAUAGU A UUUAUGA
733	AUAGUAU U UAUGAAA
734	UAGUAUU U AUGAAAU
735	AGUAUUU A UGAAAUG
745	AAAUGGU U AAGAAUU
746	AAUGGUU A AGAAUUU
752	UAAGAAU U UGGUAAA
753	AAGAAUU U GGUAAAU
757	AUUUGGU A AAUUAGU
761	GGUAAAU U AGUAUUU
762	GUAAAUU A GUAUUUA
765	AAUUAGU A UUUAUUU
767	UUAGUAU U UAUUUAA
768	UAGUAUU U AUUUAAU
769	AGUAUUU A UUUAAUG
771	UAUUUAU U UAAUGUU
772	AUUUAUU U AAUGUUA
773	UUUAUUU A AUGUUAU
778	UUAAUGU U AUGUUGU
779	UAAUGUU A UGUUGUG
783	GUUAUGU U GUGUUCU
788	GUUGUGU U CUAAUAA
789	UUGUGUU C UAAUAAA
791	GUGUUCU A AUAAAAC
794	UUCUAAU A AAACAAA
805	CAAAAAU A GACAACU

Table 12: Human IL-5 HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	GCAAAGA CUGAUGAGGCCGAAAGGCCGAA AGUGCAU
9	GGCAAAG CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
10	UGGCAAA CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
12	UUUGGCA CUGAUGAGGCCGAAAGGCCGAA AGAAAGU
13	CUUUGGC CUGAUGAGGCCGAAAGGCCGAA AAGAAAG
36	GCUCUGA CUGAUGAGGCCGAAAGGCCGAA ACGUUCU
37	GGCUCUG CUGAUGAGGCCGAAAGGCCGAA AACGUUC
38	UGGCUCU CUGAUGAGGCCGAAAGGCCGAA AAACGUU
56	AAUGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAUCC
57	AAAUGCA CUGAUGAGGCCGAAAGGCCGAA AAGCAUC
63	AAACUCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGA
64	CAAACUC CUGAUGAGGCCGAAAGGCCGAA AAUGCAG
69	GCUAGCA CUGAUGAGGCCGAAAGGCCGAA ACUCAAA
70	AGCUAGC CUGAUGAGGCCGAAAGGCCGAA AACUCAA
74	CAAGAGC CUGAUGAGGCCGAAAGGCCGAA AGCAAAC
78	GCUCCAA CUGAUGAGGCCGAAAGGCCGAA AGCUAGC
80	CAGCUCC CUGAUGAGGCCGAAAGGCCGAA AGAGCUA
91	AUACACG CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
97	GAUGGCA CUGAUGAGGCCGAAAGGCCGAA ACACGUA
104	CUGUGGG CUGAUGAGGCCGAAAGGCCGAA AUGGCAU
116	UUGUGGG CUGAUGAGGCCGAAAGGCCGAA AUUUCUG
117	CUUGUGG CUGAUGAGGCCGAAAGGCCGAA AAUUUCU
130	UUUCACC CUGAUGAGGCCGAAAGGCCGAA AUGCACU
145	CAGUGCC CUGAUGAGGCCGAAAGGCCGAA AGGUCUC
155	GAGUAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGUG
156	UGAGUAG CUGAUGAGGCCGAAAGGCCGAA AAGCAGU
157	AUGAGUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
159	CGAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAAAGC
162	GUUCGAU CUGAUGAGGCCGAAAGGCCGAA AGUAGAA
165	AGAGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAGUA
171	AUCAGCA CUGAUGAGGCCGAAAGGCCGAA AGUUCGA
179	CAUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
192	AUCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
200	GAACAGG CUGAUGAGGCCGAAAGGCCGAA AUCCUCA
201	GGAACAG CUGAUGAGGCCGAAAGGCCGAA AAUCCUC
206	GUACAGG CUGAUGAGGCCGAAAGGCCGAA ACAGGAA
207	UGUACAG CUGAUGAGGCCGAAAGGCCGAA AACAGGA
212	UUUUAUG CUGAUGAGGCCGAAAGGCCGAA ACAGGAA
216	UGAUUUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA
222	AGUUGGU CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
245	CCUGAAA CUGAUGAGGCCGAAAGGCCGAA AUUUCUU

247	UCCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUUC
248	UUCCCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUU
249	AUUCCCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
257	GUGUGCC	CUGAUGAGGCCGAAAGGCCGAA	AUUCCCU
273	ACAGUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCC
291	UCCACAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCCCU
305	UUUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUU
307	GUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCU
308	AGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGUC
316	UAAGGAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUU
319	UAUUAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUU
322	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGACAA
323	UCUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGACA
326	AUUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
334	GUCAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUU
338	GGCCGUC	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUU
380	AUUGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCC
388	GUCUAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUU
389	AGUCUAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGU
392	GGUAGUC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUU
397	UUGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUAG
409	ACCAAGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUG
410	CACCAAG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUU
411	ACACCAA	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
413	UUACACC	CUGAUGAGGCCGAAAGGCCGAA	AGAAACU
419	UGUUCAU	CUGAUGAGGCCGAAAGGCCGAA	ACACCAA
437	UUUCUAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCACU
440	AACUUUC	CUGAUGAGGCCGAAAGGCCGAA	AUUAUCC
447	UAGUCUC	CUGAUGAGGCCGAAAGGCCGAA	ACUUUCU
454	ACCAGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
462	UGCAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUU
463	CUGCAAC	CUGAUGAGGCCGAAAGGCCGAA	AACCAGU
466	UGGCUGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAACC
479	CCUCCAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUG
480	UCCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUCUUU
481	CUCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUCUU
497	GCAGUAA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
498	UGCAGUA	CUGAUGAGGCCGAAAGGCCGAA	AAUGUCC
499	CUGCAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUGUC
500	ACUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGU
531	AAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUU
538	GAAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
539	UGAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
542	UAUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
543	AUAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAUUAAG
544	UAUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAA
545	UUAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUA
549	UAAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAAA
551	GUUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUGA

554	GAAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAU
555	UGAAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA
556	CUGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAU
560	CCCUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUAAA
561	UCCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAA
573	AAAUAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUUUCC
577	CCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUACU
579	UGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUA
580	AUGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
581	UAUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAUU
588	GUGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUG
597	UCUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGUCA
598	UUCUGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUC
611	AGAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
616	UUUUAAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAU
617	AUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAUUUUA
619	AUAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUU
620	UAUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUU
625	UGAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAA
627	UCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUU
629	UAUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUU
630	AUAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAUAU
631	GAUAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAUA
636	AUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA
638	UGAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUAUCUG
644	CUUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
647	AUACUUC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUUC
653	AGGAAAA	CUGAUGAGGCCGAAAGGCCGAA	ACUUCAA
655	GGAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUACUUC
656	UGGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUACUU
657	CUGGAGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUACU
658	CCUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUAC
661	UUGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAAA
672	GUAUAUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUGC
676	AAAAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAUU
678	AAAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AUAUCAA
681	AAGAAAA	CUGAUGAGGCCGAAAGGCCGAA	AGUAUAU
682	UAAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGUAUA
683	AUAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGUAU
684	AAUAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUA
685	AAAUAAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAAGU
686	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAAAAG
688	GUUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAA
689	AGUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAA
691	UAAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAA
692	UUAAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAGA
693	GUUAAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAG
697	GAAUGUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAAA
698	AGAAUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAA

703	UUUACAG CUGAUGAGGCCGAAAGGCCGAA AUGUUAA
704	UUUUACA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
708	GACAUUU CUGAUGAGGCCGAAAGGCCGAA ACAGAAU
715	GUUAACA CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
719	UUAAGUU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
720	AUUAAGU CUGAUGAGGCCGAAAGGCCGAA AACAGAC
724	UACUAUU CUGAUGAGGCCGAAAGGCCGAA AGUUAAC
725	AUACUAU CUGAUGAGGCCGAAAGGCCGAA AAGUUAA
728	UAAAUAC CUGAUGAGGCCGAAAGGCCGAA AUUAAGU
731	UCAUAAA CUGAUGAGGCCGAAAGGCCGAA ACUAUUA
733	UUUCAUA CUGAUGAGGCCGAAAGGCCGAA AUACUAU
734	AUUUCAU CUGAUGAGGCCGAAAGGCCGAA AAUACUA
735	CAUUUCA CUGAUGAGGCCGAAAGGCCGAA AAAUACU
745	AAUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCAUUU
746	AAAUUCU CUGAUGAGGCCGAAAGGCCGAA AACCAUU
752	UUUACCA CUGAUGAGGCCGAAAGGCCGAA AUUCUUA
753	AUUUACC CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
757	ACUAAUU CUGAUGAGGCCGAAAGGCCGAA ACCAAAU
761	AAAUACU CUGAUGAGGCCGAAAGGCCGAA AUUUACC
762	UAAAUAC CUGAUGAGGCCGAAAGGCCGAA AAUUUAC
765	AAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACUAAUU
767	UUAAAUA CUGAUGAGGCCGAAAGGCCGAA AUACUAA
768	AUUAAAU CUGAUGAGGCCGAAAGGCCGAA AAUACUA
769	CAUUAAA CUGAUGAGGCCGAAAGGCCGAA AAAUACU
771	AACAUUA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
772	UAACAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAAU
773	AUAACAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAA
778	ACAACAU CUGAUGAGGCCGAAAGGCCGAA ACAUUAA
779	CACAACA CUGAUGAGGCCGAAAGGCCGAA AACAUUA
783	AGAACAC CUGAUGAGGCCGAAAGGCCGAA ACAUAAC
788	UUAUUAG CUGAUGAGGCCGAAAGGCCGAA ACACAAC
789	UUUAUUA CUGAUGAGGCCGAAAGGCCGAA AACACAA
791	GUUUUAU CUGAUGAGGCCGAAAGGCCGAA AGAACAC
794	UUUGUUU CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
805	AGUUGUC CUGAUGAGGCCGAAAGGCCGAA AUUUUUG

Table 13: Mouse IL-5 HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	cGCuCUU c CUUUGCu	253	AGGGgcU A GaCAuAC
11	uCUUcCU U UGCugAA	259	UagACAU a CUGaAgA
12	CUUcCUU U GCugAAG	269	GaAGAaU C AAACUGU
36	GAAgacU U CAGAGuC	269	GaAGAaU c AAaCugU
36	GaAgAcU u cAgAGUc	269	GAAgaAU c aAAcUgU
37	AAgacUU C AGAGuCA	287	uGGGGGU A CUGUGGA
43	UcaGaGU c AUGAgaA	301	AAAugCU A UUCcAAA
58	GGAUGCU U CUGCAcU	301	AAAugCU a uUCCaaA
59	GAUGCUU C UGCAcUU	303	AUGCuAU u CCaAaAc
59	gAUGcUU c uGcAcUU	303	AugCUAU U CcAAAAC
66	CUGCAcU U GAGUgUu	304	ugCUAUU C cAAAACc
82	UgAcucU c aGcUGUG	315	AACcUGU C aUUAAUA
91	GcUgUGU c uggGCCA	318	cUGUCaU U AAUAAAG
112	ugGAgAU U CCCAugA	319	UGUCaUU A AUAAAGA
113	gGAgAUU C CCAugAG	322	CaUUAAU A AAGAAAU
141	GAGACCU U GaCACaG	330	AAGAAAU A CAUUGAC
141	GAgACcU U GaCAcAg	334	AAUACAU U GACcGCC
158	gUCcgCU C AcCGAgC	334	AAUaCaU u GACcgCC
167	cCGAgCU C UGuUGAc	384	AggCAgU U CCUgGAu
196	UGAGGcU U CCUGUcC	385	ggCAgUU C CUgGAuU
197	GAGGcUU C CUGUcCC	393	CUgGAuU A CCUGCAA
197	gAGGCuU c CUGuCcC	405	CAAGAGU U cCUUGGU
202	UUCCUGU c CCUacuC	406	AAGAGUU c CUUGGUG
202	UUCCUGU c CcUAcuc	409	AGUUcCU U GGUGUgA
206	UGUCccU a cuCaUAA	481	UcaCAAU u UAAgUUA
212	UACUCAU a aAAaUCa	482	cAcAAUU U AAgUUaA
212	UacuCAU A AAAAUCA	483	AcAAUUU A AgUUaAa
218	UaaAaaU c aCcAGCU	483	AcAAUuU a aGUUAAa
218	UAAAAAU C ACCAgCU	495	AAAUUgU c AAcAgAU
218	uAAAAAU c acCAgCU	553	GCUGuuU c CaUuUAU
232	uaUGCAU U GGaGAAA	557	UuUcCAU U UauaUUU
241	gAGAAAU C UUUCAGG	564	UUauAuU u aUgUCCU
241	gAgAaAU c UUucAGG	564	UUAuaUU u AugUcCU
241	gagAAAU c UUUCAGG	565	uaUAUUU a ugUCCuG
241	gAgAaAU c UUUCAGg	565	UAUAuUU a UgUCcUg
243	gaAAucU U UCAGgGg	569	UUuAUGU c cUGUaGU
243	GAAAUCU U UCAGGGg	569	uUUAUGU c cUGUagU
244	AAAUCUU U CAGGGgc	613	AAAGuGU u uaaCCUU
245	AAUCUUU C AGGGgcU	614	AAgUGuU u aACCcUUU

620	UUAACcU u uUuGUAU
793	caAGgCU u UGuGcAU
816	CUGagUU a UACUCcc
818	GAguUAU a cUCCcuC
825	ACUcCcU c CccCUCA
825	aCUccCU c CcCcUCa
839	AuCcucU U cGUUGCA
840	uCcucUU c GUUGCAu
863	cAAgUAU U cCAGGCu
864	AAgUAUU c CAGGCug
864	AAGUAUU c caggCug
913	gAaCUCU U GGucCaG
917	UcUuggU c CAGAuGG
957	UUagcAU c CUUUcUc
960	GCAuccU u UcUcCuA
960	GcaUcCU u uCUCcUa
962	AUcCuuU c UCcUaGC
975	gcccCUU u AgAUAgA
987	aGaUGAU A cuuAAUG
990	UGAuACU u AAugacU
1000	UGACuCU c UugCuGA
1027	CgggGCU U cCUgCUC
1034	UCCUGcU C CUaUcuA
1037	UgcUCcU A UcUAACU
1039	cUccuAU c UAACUUC
1039	cUCcUAU c UAACUUc
1041	CcUAUcU A ACUUcAa
1051	UUcAAuU U AAuAccC
1148	uGAcUUU u cUuaUGU
1213	GCUgGaU u UUGGAaa
1213	gcUGGAU u uUgGAAA
1214	cugGAUU U UGGAaaA
1215	ugGAUUU U GGAaaAG
1234	gGGACAU c UccuUGC
1236	GACAUcU c cuUGCAG
1275	ugGGCCU U AcUUcUC
1276	gGGCCUU A cUUcUCc
1280	CUUAcUU c UCcgUgU
1298	UgAACUU a AGAaGcA
1310	gcAAAGU a aAuACcA
1310	GCAAAgU a aAUAcca
1310	GcaAAgU a AAUAccA
1350	AAAGCAU A AAAUggU
1358	AAAUGGU U ggGAugU
1370	UgUuaUU C AGgUAUC
1375	UUCAGgU A UCAGggU
1377	CAGgUAU C AGggUCA
1383	UCAGggU C AcUGgAG
1405	cccCAgU U UACUcCA
1407	cCAgUUU A CUcCAGg
1407	ccAgUUU a CUCCAGG
1410	gUUUaCU C CAGGaAA
1434	AUgCUUU U aUuUaAU
1434	aUgcUuU U AUUUAAu
1434	aUgcuUU u AuUUAAU
1435	UgCUUUU a UuUaAUU
1435	ugcUUUU a uUUAaUU
1438	UuUUAUU U AAuUcug
1438	uUUUAUU U AAUucUg
1439	UUUAUUU A AUucUgU
1443	UUUaAuU c UGuaAGa
1447	AUUCUGU A AgAUGUu
1458	ugUUcaU a UUAUUUA
1458	ugUUcAU A uUAUUUA
1460	UucAUAU u AUUUAug
1461	UcAUAuU A UUUAUGA
1463	AUAuUAU U UAUGAug
1475	AuGgAUU c aGUAAgU
1479	AUUcaGU A AgUUAaU
1483	aGuAAGU u AAUAUUU
1483	aGUAAgU U AaUAUUU
1484	GUAAgUU A aUAUUUA
1487	agUUAAU a UUuAuUA
1487	AgUUAaU A UUUAUUa
1489	UUAAUaU U uAuUAcA
1489	UUAAuAU u UAUUaCA
1489	UUAaUAU U UAUUacA
1490	UAAUaUU u AuUAcAc
1490	UAaUAUU U AUuAcAc
1490	UAaUAUU U AUUacAc
1491	AAUAUUU a uuaCAcg
1491	AAUAUuU a UuAcAcg
1491	AaUAUUU A UuAcAcG
1491	AaUAUUU A UUacAcG
1494	AUuUAUU a CAcgUAU
1502	cACGUaU A UaauAUu
1502	cAcgUAU a UAAUaUU
1507	AUAUAaU a UUcUaaU
1509	AUAAuAU U CUaAuAA
1509	aUaaUaU U CUAAUAA
1510	UAAuAUU C UaAuAAa
1510	UAAuAUU C UaauAAA
1510	UAAuAuU c UaaUAAA
1510	UaaUaUU C UAAUAAA
1512	aUaUUCU A AUAAAgC
1515	UUCUAAU A AAgCAgA

Table 14: Human IL-6 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
86	UACACGUA AGAA GCUCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGAGCU GCC UACGUGUA
151	GAGUAGAA AGAA GUGCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGCACU GCU UUCUACUC
172	UGGCUAUC AGAA GAGUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACUCU GCU GAUAGCCA
203	UGUACAGG AGAA GGAAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAUUCCU GUU CCUGUACA

Table 15: Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	AGCUGAGA AGAA GAACAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGUUCU GAC UCUCAGCU
83	CCAGACAC AGAA GAGAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACUCUCA GCU GUGUCUGG
147	GAGCGGAC AGAA GUGUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGACACA GCU GUCCGCUC
150	GGUGAGCG AGAA GCUGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CACAGCU GUC CGCUCACC
154	GCUCGGUG AGAA GACAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUGUCC GCU CACCGAGC
168	UGCUUGUC AGAA GAGCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCUCU GUU GACAAGCA
199	UGAGUAGG AGAA GGAAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUUCCU GUC CCUACUCA
274	CCCCCACG AGAA GUUUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCAAACU GUC CGUGGGGG
381	AAUCCAGG AGAA GCCUCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGAGGCA GUU CCUGGAUU
454	CACCAUGG AGAA GCUCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGAGCU GCU CCAUGGUG
499	GUUUUUGC AGAA GUUGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUCAACA GAU GCAAAAAC
548	UAAAUGGA AGAA GCAUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUAUGCU GUU UCCAUUUA
701	GCAGGAGG AGAA GAAAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUUUCU GAU CCUCCUGC
710	GAAGAGGA AGAA GGAGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCUCCU GCC UCCUCUUC
870	AGUUCAAA AGAA GCCUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCAGGCU GAC UUUGAACU
919	CUGCGUCC AGAA GGACCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGUCCA GAU GGACGCAG
1030	UAGAUAGG AGAA GGAAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUUCCU GCU CCUAUCUA
1170	AUGGCACA AGAA GAUUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGAAUCA GAC UGUGCCAU
1205	CAAAAUCC AGAA GCUCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGAGCA GCU GGAUUUUG
1402	CUGGAGUA AGAA GGGGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCCCCA GUU UACUCCAG
1421	AAGCAUAC AGAA GUUUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAAACA GAU GUAUGCUU

Table 16 : Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	AGCUGAGA AGAA GAACAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGUUCU GAC UCUCAGCU
83	CCAGACAC AGAA GAGAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACUCUCA GCU GUGUCUGG
147	GAGCGGAC AGAA GUGUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGACACA GCU GUCCGCUC
150	GGUGAGCG AGAA GCUGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CACAGCU GUC CGCUCACC
154	GCUCGGUG AGAA GACAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUGUCC GCU CACCGAGC
168	UGCUUGUC AGAA GAGCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCUCU GUU GACAAGCA
199	UGAGUAGG AGAA GGAAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUUCCU GUC CCUACUCA
274	CCCCCACG AGAA GUUUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCAAACU GUC CGUGGGGG
381	AAUCCAGG AGAA GCCUCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGAGGCA GUU CCUGGAUU
454	CACCAUGG AGAA GCUCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGAGCU GCU CCAUGGUG
499	GUUUUUGC AGAA GUUGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUCAACA GAU GCAAAAAC
548	UAAAUGGA AGAA GCAUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUAUGCU GUU UCCAUUUA
701	GCAGGAGG AGAA GAAAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUUUCU GAU CCUCCUGC
710	GAAGAGGA AGAA GGAGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCUCCU GCC UCCUCUUC
870	AGUUCAAA AGAA GCCUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCAGGCU GAC UUUGAACU
919	CUGCGUCC AGAA GGACCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGUCCA GAU GGACGCAG
1030	UAGAUAGG AGAA GGAAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUUCCU GCU CCUAUCUA
1170	AUGGCACA AGAA GAUUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGAAUCA GAC UGUGCCAU
1205	CAAAAUCC AGAA GCUCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGAGCA GCU GGAUUUUG
1402	CUGGAGUA AGAA GGGGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCCCCA GUU UACUCCAG
1421	AAGCAUAC AGAA GUUUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAAACA GAU GUAUGCUU

Table 17
Mouse *rel A* HH Target sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
19	AAUGGCU a caCaGgA	467	cCAGGCU c cuguUCg
22	aGCUCcU a cGUgGUG	469	AaGCcAU u AGcCAGC
26	CcUCcaU u GcGgACa	473	UuUgAGU C AGauCAg
93	GAuCUGU U uCCCCUC	481	AGCGaAU C CAGACCA
94	AuCUGUU u CCCCUCA	501	AACCCCU U uCAcGUU
100	UuCCCCU C AUCUUuC	502	ACCCCUU u CAcGUUC
103	CCCUCAU C UUuCCcu	508	UuCAcGU U CCUAUAG
105	CUCAUCU U uCCcuCA	509	uCAcGUU C CUAUAGA
106	UCAUCUU u CCcuCAG	512	cGUUCCU A UAGAgGA
129	CAGGCuU C UGGgCCu	514	UUCCUAU A GAgGAGC
138	GGgCCuU A UGUGGAG	534	GGGGACU A uGACuUG
148	UGGAGAU C AUcGAaC	556	UGCGcCU C UGCUUCC
151	AGAUCAU c GAaCAGC	561	CUCUGCU U CCAGGUG
180	AUGCGaU U CCGCUAu	562	UCUGCUU C CAGGUGA
181	UGCGaUU C CGCUAuA	585	aAgCCAU u AGcCAGc
186	UUCCGCU A uAAaUGC	598	GGCCCCU C CuCCUGa
204	GGGCGCU C aGCGGGC	613	CcCCUGU C CUcuCaC
217	GCAGuAU u CCuGGCG	616	CUGUCCU c uCaCAUC
239	CACAGAU A CCACCAA	617	gucCCUU C CUCAgCC
262	CCACCAU C AAGAUCA	620	CCUUCCU C AgCCaug
268	UCAAGAU C AAUGGCU	623	UCCUgcU u CCAUCUc
276	AAUGGCU A CACAGGA	628	AUCCgAU u UUUGAuA
301	UuCGaAU C UCCCUGG	630	CCgAUuU U UGAuAAc
303	CGaAUCU C CCUGGUC	631	CgAUuUU U GAuAAcC
310	CCCUGGU C ACCAAGG	638	UGgCcAU u GUGuuCC
323	GGcCCCU C CUCcuga	661	CCGAGCU C AAGAUCU
326	uCCaCCU C ACCGGCC	667	UCAAGAU C UGCCGAG
335	CCGGCCU C AuCCaCA	687	CGgAACU C UGGgAGC
349	AuGAaCU U GUgGGgA	700	GCUGCCU C GGUGGGG
352	AGaUcaU c GaAcAGc	715	AUGAGAU C UUCuUgC
375	GAUGGCU a CUAUGAG	717	GAGAUCU U CuUgCUG
376	AUGGucU C UccGgaG	718	AGAUCUU C uUgCUGU
378	GGCUaCU A UGAGGCU	721	UucUCCU c CauUGcG
391	CUGAcCU C UGCCCaG	751	AaGACAU U GAGGUGU
409	GCaGuAU C CAuAGcU	759	GAGGUGU A UUUCACG
416	CCgCAGU a UCCAuAg	761	GGUGUAU U UCACGGG
417	CAuAGcU U CCAGAAC	762	GUGUAUU U CACGGGA
418	AuAGcUU C CAGAACC	763	UGUAUUU C ACGGGAC
433	UGGGgAU C CAGUGUG	792	CGAGGCU C CUUUUCu
795	GGCUCCU U UUCuCAA	1167	GAUGAGU U UuCCcCC
796	GCUCCUU U UCuCAAG	1168	AUGAGUU U uCCcCCA
797	CUCCUUU U CuCAAGC	1169	UGAGUUU u CCcCCAU
798	UCCUUUU C uCAAGCU	1182	AUGcUGU U aCCaUCa
829	UGGCCAU U GUGUUCC	1183	UGcUGUU a CCaUCaG

834	AUUGUGU U CCGGACu	1184	GGccccU C CUcCUGa
835	UUGUGUU C CGGACuC	1187	GUccCuU c CUcaGCc
845	GACuCCU C CgUACGC	1188	UUaCCaU C aGGGCAG
849	CCUCCgU A CGCcGAC	1198	GGgAGuU u AGuCuGa
872	cCAGGCU C CUGUuCG	1209	CAGcCCU a caCCUUc
883	UuCGaGU C UCCAUGC	1215	cuGGCCU U aGCaCCG
885	CGaGUCU C CAUGCAG	1229	GGuCCCU u CCucAGc
905	GCGGCCU U CuGAuCG	1237	CCCAgcU C CUGCCCC
906	CGGCCUU C uGAuCGc	1250	CCAGcCU C CAGgCuC
919	GcGAGCU C AGUGAGC	1268	CCCaGCU C CuGCCcc
936	AUGGAgU U CCAGUAC	1279	CCAUGGU c cCuuCcu
937	UGGAgUU C CAGUACu	1281	gUGGgcU C AGCUgcG
942	UUCCAGU A CuUGCCA	1286	AUgAGuU u UccCCCA
953	GCCucAU c CAcAuGA	1309	CuCCUGU u CgAGUCu
962	AGAuGAU C GcCACCG	1315	cCCCAGU u CUAaCCC
965	CagUacU u gCCaGAc	1318	CAGUuCU A aCCCCgG
973	ACCGGAU U GAaGAGA	1331	gGGuCCU C CcCAGuC
986	GAgACcU u cAAGagu	1334	CuuUuCU C AaGCUGa
996	AGGACcU A UGAGACC	1389	ACGCUGU C gGAaGCC
1005	GAGACCU U CAAGAGu	1413	CUGCAGU U UGAUGcU
1006	AGACCUU C AAGAGuA	1414	UGCAGUU U GAUGcUG
1015	AGAGuAU C AUGAAGA	1437	GGGGCCU U GCUUGGC
1028	GAAGAGU C CUUUCAa	1441	CCUUGCU U GGCAACA
1031	GAGUCCU U UCAauGG	1467	GgaGUGU U CACAGAC
1032	AGUCCUU U CAauGGA	1468	gaGUGUU C ACAGACC
1033	GUCCUUU C AauGGAC	1482	CUGGCAU C uGUgGAC
1058	CCGGCCU C CAaCcCG	1486	CuUCgGU a GggAACU
1064	UaCACCU u GAucCAa	1494	GACAACU C aGAGUUU
1072	GgCGuAU U GCUGUGC	1500	UCaGAGU U UCAGCAG
1082	UGUGCCU a CCCGaAa	1501	CaGAGUU U CAGCAGC
1083	aaGCCUU C CCGaAGu	1502	aGAGUUU C AGCAGCU
1092	CGaAaCU C AaCUUCU	1525	gGuGCAU c CCUGUGu
1097	CUCAaCU U CUGUCCC	1566	AUGGAGU A CCCUGAa
1098	UCAaCUU C UGUCCCC	1577	UGAaGCU A UAACUCG
1102	CUUCUGU C CCCAAGC	1579	AaGCUAU A ACUCGCC
1125	CAGCCCU A caCCUUc	1583	UAUAACU C GCCUgGU
1127	GCCaUAU a gCcUUAC	1588	CUCuCCU A GaGAggG
1131	cAUCCCU c agCacCA	1622	CCCAGCU C CUGCcCC
1132	AcaCCUU c cCagCAU	1628	UCCUGCU u CggUaGG
1133	UCCaUcU c CagCuUC	1648	CGGGGCU u CCCAAUG
1137	UUUACuU u AgCgCgc	1660	cUGaCCU C ugccCAG
1140	cCagCAU C CCUcAGC	1663	cuCUgCU U cCAGGuG
1153	GCACCAU C AACUuUG	1664	uCUgCUU c CAGGuGA
1158	AUCAACU u UGAUGAG	1665	CUCgcUU u cGGAGgU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GCGGACA		

1704	AUGGACU U CUCuGCu
1705	UGGACUU C UCuGCuC
1707	GACUUCU C uGCuCUu
1721	uuUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGu
1734	AGCUCCU A AGGuGcU
1754	CaGugCU C CCaAGAG

Table 18
Human *rel A* HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
19	AAUGGCU C GUCUGUA	467	GCAGGCU A UCAGUCA
22	GGCUCGU C UGUAGUG	469	AGGCUAU C AGUCAGC
26	CGUCUGU A GUGCACG	473	UAUCAGU C AGCGCAU
93	GAACUGU U CCCCCUC	481	AGCGCAU C CAGACCA
94	AACUGUU C CCCCUCA	501	AACCCCU U CCAAGUU
100	UCCCCCU C AUCUUCC	502	ACCCCUU C CAAGUUC
103	CCCUCAU C UUCCCGG	508	UCCAAGU U CCUAUAG
105	CUCAUCU U CCCGGCA	509	CCAAGUU C CUAUAGA
106	UCAUCUU C CCGGCAG	512	AGUUCCU A UAGAAGA
129	CAGGCCU C UGGCCCC	514	UUCCUAU A GAAGAGC
138	GGCCCCU A UGUGGAG	534	GGGGACU A CGACCUG
148	UGGAGAU C AUUGAGC	556	UGCGGCU C UGCUUCC
151	AGAUCAU U GAGCAGC	561	CUCUGCU U CCAGGUG
180	AUGCGCU U CCGCUAC	562	UCUGCUU C CAGGUGA
181	UGCGCUU C CGCUACA	585	GACCCAU C AGGCAGG
186	UUCCGCU A CAAGUGC	598	GGCCCCU C CGCCUGC
204	GGGCGCU C CGCGGGC	613	CGCCUGU C CUUCCUC
217	GCAGCAU C CCAGGCG	616	CUGUCCU U CCUCAUC
239	CACAGAU A CCACCAA	617	UGUCCUU C CUCAUCC
262	CCACCAU C AAGAUCA	620	CCUUCCU C AUCCCAU
268	UCAAGAU C AAUGGCU	623	UCCUCAU C CCAUCUU
276	AAUGGCU A CACAGGA	628	AUCCCAU C UUUGACA
301	UGCGCAU C UCCCUGG	630	CCCAUCU U UGACAAU
303	CGCAUCU C CCUGGUC	631	CCAUCUU U GACAAUC
310	CCCUGGU C ACCAAGG	638	UGACAAU C GUGCCCC
323	GGACCCU C CUCACCG	661	CCGAGCU C AAGAUCU
326	CCCUCCU C ACCGGCC	667	UCAAGAU C UGCCGAG
335	CCGGCCU C ACCCCCA	687	CGAAACU C UGGCAGC
349	ACGAGCU U GUAGGAA	700	GCUGCCU C GGUGGGG
352	AGCUUGU A GGAAAGG	715	AUGAGAU C UUCCUAC
375	GAUGGCU U CUAUGAG	717	GAGAUCU U CCUACUG
376	AUGGCUU C UAUGAGG	718	AGAUCUU C CUACUGU
378	GGCUUCU A UGAGGCU	721	UCUUCCU A CUGUGUG
391	CUGAGCU C UGCCCGG	751	AGGACAU U GAGGUGU
409	GCUGCAU C CACAGUU	759	GAGGUGU A UUUCACG
416	CCACAGU U UCCAGAA	761	GGUGUAU U UCACGGG
417	CACAGUU U CCAGAAC	762	GUGUAUU U CACGGGA
418	ACAGUUU C CAGAACC	763	UGUAUUU C ACGGGAC
433	UGGGAAU C CAGUGUG	792	CGAGGCU C CUUUUCG
795	GGCUCCU U UUCGCAA	1167	GAUGAGU U UCCCACC
796	GCUCCUU U UCGCAAG	1168	AUGAGUU U CCCACCA
797	CUCCUUU U CGCAAGC	1169	UGAGUUU C CCACCAU
798	UCCUUUU C GCAAGCU	1182	AUGGUGU U UCCUUCU
829	UGGCCAU U GUGUUCC	1183	UGGUGUU U CCUUCUG
834	AUUGUGU U CCGGACC	1184	GGUGUUU C CUUCUGG

835	UUGUGUU C CGGACCC
845	GACCCCU C CCUACGC
849	CCUCCCU A CGCAGAC
872	GCAGGCU C CUGUGCG
883	UGCGUGU C UCCAUGC
885	CGUGUCU C CAUGCAG
905	GCGGCCU U CCUACCG
906	CGGCCUU C CGACCGG
919	GGGAGCU C AGUGAGC
936	AUGGAAU U CCAGUAC
937	UGGAAUU C CAGUACC
942	UUCCAGU A CCUGCCA
953	GCCAGAU A CAGACGA
962	AGACGAU C GUCACCG
965	CGAUCGU C ACCGGAU
973	ACCGGAU U GAGGAGA
986	GAAACGU A AAAGGAC
996	AGGACAU A UGAGACC
1005	GAGACCU U CAAGAGC
1006	AGACCUU C AAGAGCA
1015	AGAGCAU C AUGAAGA
1028	GAAGAGU C CUUUCAG
1031	GAGUCCU U UCAGCGG
1032	AGUCCUU U CAGCGGA
1033	GUCCUUU C AGCGGAC
1058	CCGGCCU C CACCUCG
1064	UCCACCU C GACGCAU
1072	GACGCAU U GCUGUGC
1082	UGUGCCU U CCCGCAG
1083	GUGCCUU C CCGCAGC
1092	CGCAGCU C AGCUUCU
1097	CUCAGCU U CUGUCCC
1098	UCAGCUU C UGUCCCC
1102	CUUCUGU C CCCAAGC
1125	CAGCCCU A UCCCUUU
1127	GCCCUAU C CCUUUAC
1131	UAUCCCU U UACGUCA
1132	AUCCCUU U ACGUCAU
1133	UCCCUUU A CGUCAUC
1137	UUUACGU C AUCCCUG
1140	ACGUCAU C CCUGAGC
1153	GCACCAU C AACUAUG
1158	AUCAACU A UGAUGAG
1680	GAAGACU U CUCCUCC
1681	AAGACUU C UCCUCCA
1683	GACUUCU C CUCCAUU
1686	UUCUCCU C CAUUGCG
1690	CCUCCAU U GCGGACA
1704	AUGGACU U CUCAGCC
1187	GUUUCCU U CUGGGCA
1188	UUUCCUU C UGGGCAG
1198	GGCAGAU C AGCCAGG
1209	CAGGCCU C GGCCUUG
1215	UCGGCCU U GGCCCCG
1229	GGCCCCU C CCCAAGU
1237	CCCAAGU C CUGCCCC
1250	CCAGGCU C CAGCCCC
1268	CCCUGCU C CAGCCAU
1279	CCAUGGU A UCAGCUC
1281	AUGGUAU C AGCUCUG
1286	AUCAGCU C UGGCCCA
1309	CCCCUGU C CCAGUCC
1315	UCCCAGU C CUAGCCC
1318	CAGUCCU A GCCCCAG
1331	AGGCCCU C CUCAGGC
1334	CCCUCCU C AGGCUGU
1389	ACGCUGU C AGAGGCC
1413	CUGCAGU U UGAUGAU
1414	UGCAGUU U GAUGAUG
1437	GGGGCCU U GCUUGGC
1441	CCUUGCU U GGCAACA
1467	GCUGUGU U CACAGAC
1468	CUGUGUU C ACAGACC
1482	CUGGCAU C CGUCGAC
1486	CAUCCGU C GACAACU
1494	GACAACU C CGAGUUU
1500	UCCGAGU U UCAGCAG
1501	CCGAGUU U CAGCAGC
1502	CGAGUUU C AGCAGCU
1525	AGGGCAU A CCUGUGG
1566	AUGGAGU A CCCUGAG
1577	UGAGGCU A UAACUCG
1579	AGGCUAU A ACUCGCC
1583	UAUAACU C GCCUAGU
1588	CUCGCCU A GUGACAG
1622	CCCAGCU C CUGCUCC
1628	UCCUGCU C CACUGGG
1648	CGGGGCU C CCCAAUG
1660	AUGGCCU C CUUUCAG
1663	GCCUCCU U UCAGGAG
1664	CCUCCUU U CAGGAGA
1665	CUCCUUU C AGGAGAU

1705	UGGACUU C UCAGCCC
1707	GACUUCU C AGCCCUG
1721	GCUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGG
1734	AGCUCCU A AGGGGGU
1754	CUGCCCU C CCCAGAG

Table 19

Mouse *rel A* HH Ribozyme Sequences

nt. Sequence	HH Ribozyme Sequence
19	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
22	CACCACG CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
26	UGUCCGC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
93	GAGGGGA CUGAUGAGGCCGAAAGGCCGAA ACAGAUC
94	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AACAGAU
100	GAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGAA
103	AGGGAAA CUGAUGAGGCCGAAAGGCCGAA AUGAGGG
105	UGAGGGA CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
106	CUGAGGG CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
129	AGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAGCCUG
138	CUCCACA CUGAUGAGGCCGAAAGGCCGAA AAGGCCC
148	GUUCGAU CUGAUGAGGCCGAAAGGCCGAA AUCUCCA
151	GCUGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
180	AUAGCGG CUGAUGAGGCCGAAAGGCCGAA AUCGCAU
181	UAUAGCG CUGAUGAGGCCGAAAGGCCGAA AAUCGCA
186	GCAUUUA CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
204	GCCCGCU CUGAUGAGGCCGAAAGGCCGAA AGCGCCC
217	CGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACUGC
239	UUGGUGG CUGAUGAGGCCGAAAGGCCGAA AUCUGUG
262	UGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGG
268	AGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
276	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
301	CCAGGGA CUGAUGAGGCCGAAAGGCCGAA AUUCGAA
303	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AGAUUCG
310	CCUUGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
323	UCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
326	GGCCGGU CUGAUGAGGCCGAAAGGCCGAA AGGUGGA
335	UGUGGAU CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
349	UCCCCAC CUGAUGAGGCCGAAAGGCCGAA AGUUCAU
352	GCUGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
375	CUCAUAG CUGAUGAGGCCGAAAGGCCGAA AGCCAUC
376	CUCCGGA CUGAUGAGGCCGAAAGGCCGAA AGACCAU
378	AGCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUAGCC
391	CUGGGCA CUGAUGAGGCCGAAAGGCCGAA AGGUCAG
409	AGCUAUG CUGAUGAGGCCGAAAGGCCGAA AUACUGC
416	CUAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUGCGG
417	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA AGCUAUG
418	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCUAU
433	CACACUG CUGAUGAGGCCGAAAGGCCGAA AUCCCCA
467	CGAACAG CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
469	GCUGGCU CUGAUGAGGCCGAAAGGCCGAA AUGGCUU
473	CUGAUCU CUGAUGAGGCCGAAAGGCCGAA ACUCAAA
481	UGGUCUG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU

501	AACGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUU
502	GAACGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGGU
508	CUAUAGG	CUGAUGAGGCCGAAAGGCCGAA	ACGUGAA
509	UCUAUAG	CUGAUGAGGCCGAAAGGCCGAA	AACGUGA
512	UCCUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGAACG
514	GCUCCUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGAA
534	CAAGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCCC
556	GGAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGCA
561	CACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
562	UCACCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
585	GCUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCUU
598	UCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
613	GUGAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGG
616	GAUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGACAG
617	GGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAC
620	CAUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
623	GAGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGA
628	UAUCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGGAU
630	GUUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCGG
631	GGUUAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCG
638	GGAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCA
661	AGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCGG
667	CUCGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
687	GCUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUCCG
700	CCCCACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
715	GCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAU
717	CAGCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCUC
718	ACAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCU
721	CGCAAUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAA
751	ACACCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
759	CGUGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCUC
761	CCCGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUACACC
762	UCCCGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUACAC
763	GUCCCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
792	AGAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCG
795	UUGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCC
796	CUUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGC
797	GCUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAG
798	AGCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGA
829	GGAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCA
834	AGUCCGG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAU
835	GAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
845	GCGUACG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGUC
849	GUCGGCG	CUGAUGAGGCCGAAAGGCCGAA	ACGGAGG
872	CGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCG
883	GCAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGAA
885	CUGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUCG
905	CGAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGC
906	GCGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCG

919 GCUCACU CUGAUGAGGCCGAAAGGCCGAA AGCUCGC
936 GUACUGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAU
937 AGUACUG CUGAUGAGGCCGAAAGGCCGAA AACUCCA
942 UGGCAAG CUGAUGAGGCCGAAAGGCCGAA ACUGGAA
953 UCAUGUG CUGAUGAGGCCGAAAGGCCGAA AUGAGGC
962 CGGUGGC CUGAUGAGGCCGAAAGGCCGAA AUCAUCU
965 GUCUGGC CUGAUGAGGCCGAAAGGCCGAA AGUACUG
973 UCUCUUC CUGAUGAGGCCGAAAGGCCGAA AUCCGGU
986 ACUCUUG CUGAUGAGGCCGAAAGGCCGAA AGGUCUC
996 GGUCUCA CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
1005 ACUCUUG CUGAUGAGGCCGAAAGGCCGAA AGGUCUC
1006 UACUCUU CUGAUGAGGCCGAAAGGCCGAA AAGGUCU
1015 UCUUCAU CUGAUGAGGCCGAAAGGCCGAA AUACUCU
1028 UUGAAAG CUGAUGAGGCCGAAAGGCCGAA ACUCUUC
1031 CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AGGACUC
1032 UCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAGGACU
1033 GUCCAUU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
1058 CGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
1064 UUGGAUC CUGAUGAGGCCGAAAGGCCGAA AGGUGUA
1072 GCACAGC CUGAUGAGGCCGAAAGGCCGAA AUACGCC
1082 UUUCGGG CUGAUGAGGCCGAAAGGCCGAA AGGCACA
1083 ACUUCGG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
1092 AGAAGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCG
1097 GGGACAG CUGAUGAGGCCGAAAGGCCGAA AGUUGAG
1098 GGGGACA CUGAUGAGGCCGAAAGGCCGAA AAGUUGA
1102 GCUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
1125 GAAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
1127 GUAAGGC CUGAUGAGGCCGAAAGGCCGAA AUAUGGC
1131 UGGUGCU CUGAUGAGGCCGAAAGGCCGAA AGGGAUG
1132 AUGCUGG CUGAUGAGGCCGAAAGGCCGAA AAGGUGU
1133 GAAGCUG CUGAUGAGGCCGAAAGGCCGAA AGAUGGA
1137 GCGCGCU CUGAUGAGGCCGAAAGGCCGAA AAGUAAA
1140 GCUGAGG CUGAUGAGGCCGAAAGGCCGAA AUGCUGG
1153 CAAAGUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGC
1158 CUCAUCA CUGAUGAGGCCGAAAGGCCGAA AGUUGAU
1167 GGGGGAA CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
1168 UGGGGGA CUGAUGAGGCCGAAAGGCCGAA AACUCAU
1169 AUGGGGG CUGAUGAGGCCGAAAGGCCGAA AAACUCA
1182 UGAUGGU CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
1183 CUGAUGG CUGAUGAGGCCGAAAGGCCGAA AACAGCA
1184 UCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
1187 GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
1188 CUGCCCU CUGAUGAGGCCGAAAGGCCGAA AUGGUAA
1198 UCAGACU CUGAUGAGGCCGAAAGGCCGAA AACUCCC
1209 GAAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
1215 CGGUGCU CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
1229 GCUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGGACC
1237 GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
1250 GAGCCUG CUGAUGAGGCCGAAAGGCCGAA AGGCUGG

1268	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1279	AGGAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGG
1281	CGCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCCAC
1286	UGGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AACUCAU
1309	AGACUCG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAG
1315	GGGUUAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGG
1318	CCGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGAACUG
1331	GACUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGACCC
1334	UCAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAG
1389	GGCUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACAGCGU
1413	AGCAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAG
1414	CAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AACUGCA
1437	GCCAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1441	UGUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGG
1467	GUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	ACACUCC
1468	GGUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AACACUC
1482	GUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
1486	AGUUCCC	CUGAUGAGGCCGAAAGGCCGAA	ACCGAAG
1494	AAACUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUC
1500	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGA
1501	GCUGCUG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUG
1502	AGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
1525	ACACAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGCACC
1566	UUCAGGG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAU
1577	CGAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCA
1579	GGCGAGU	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUU
1583	ACCAGGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUA
1588	CCCUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAG
1622	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
1628	CCUACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGA
1648	CAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCG
1660	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
1663	CACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1664	UCACCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
1665	ACCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAGCGAG
1680	GGAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUC
1681	UGGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUU
1683	AAUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
1686	CGCAAUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAA
1690	UGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGG
1704	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAU
1705	GAGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCA
1707	AAGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUC
1721	CUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	ACUCAAA
1726	AGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAC
1731	ACCUUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAU
1734	AGCACCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCU
1754	CUCUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCACUG

Table 20
Human *rel A* HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
19	UACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
22	CACUACA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
26	CGUGCAC CUGAUGAGGCCGAAAGGCCGAA ACAGACG
93	GAGGGGG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
94	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AACAGUU
100	GGAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
103	CCGGGAA CUGAUGAGGCCGAAAGGCCGAA AUGAGGG
105	UGCCGGG CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
106	CUGCCGG CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
129	GGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
138	CUCCACA CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
148	GCUCAAU CUGAUGAGGCCGAAAGGCCGAA AUCUCCA
151	GCUGCUC CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
180	GUAGCGG CUGAUGAGGCCGAAAGGCCGAA AGCGCAU
181	UGUAGCG CUGAUGAGGCCGAAAGGCCGAA AAGCGCA
186	GCACUUG CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
204	GCCCGCG CUGAUGAGGCCGAAAGGCCGAA AGCGCCC
217	CGCCUGG CUGAUGAGGCCGAAAGGCCGAA AUGCUGC
239	UUGGUGG CUGAUGAGGCCGAAAGGCCGAA AUCUGUG
262	UGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGG
268	AGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
276	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
301	CCAGGGA CUGAUGAGGCCGAAAGGCCGAA AUGCGCA
303	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
310	CCUUGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
323	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AGGGUCC
326	GGCCGGU CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
335	UGGGGGU CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
349	UUCCUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCGU
352	CCUUUCC CUGAUGAGGCCGAAAGGCCGAA ACAAGCU
375	CUCAUAG CUGAUGAGGCCGAAAGGCCGAA AGCCAUC
376	CCUCAUA CUGAUGAGGCCGAAAGGCCGAA AAGCCAU
378	AGCCUCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
391	CCGGGCA CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
409	AACUGUG CUGAUGAGGCCGAAAGGCCGAA AUGCAGC
416	UUCUGGA CUGAUGAGGCCGAAAGGCCGAA ACUGUGG
417	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA AACUGUG
418	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAACUGU
433	CACACUG CUGAUGAGGCCGAAAGGCCGAA AUUCCCA
467	UGACUGA CUGAUGAGGCCGAAAGGCCGAA AGCCUGC
469	GCUGACU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
473	AUGCGCU CUGAUGAGGCCGAAAGGCCGAA ACUGAUA
481	UGGUCUG CUGAUGAGGCCGAAAGGCCGAA AUGCGCU
501	AACUUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUU

502	GAACUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGGGU
508	CUAUAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGA
509	UCUAUAG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGG
512	UCUUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGAACU
514	GCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AUAGGAA
534	CAGGUCG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCCC
556	GGAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCA
561	CACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
562	UCACCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
585	CCUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUC
598	GCAGGCG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
613	GAGGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCG
616	GAUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGACAG
617	GGAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGACA
620	AUGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
623	AAGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGA
628	UGUCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAU
630	AUUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGG
631	GAUUGUC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGG
638	GGGGCAC	CUGAUGAGGCCGAAAGGCCGAA	AUUGUCA
661	AGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCGG
667	CUCGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
687	GCUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCG
700	CCCCACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
715	GUAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAU
717	CAGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCUC
718	ACAGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCU
721	CACACAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
751	ACACCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
759	CGUGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCUC
761	CCCGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUACACC
762	UCCCGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUACAC
763	GUCCCGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
792	CGAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUCG
795	UUGCGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCC
796	CUUGCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGC
797	GCUUGCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAG
798	AGCUUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGA
829	GGAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCA
834	GGUCCGG	CUGAUGAGGCCGAAAGGCCGAA	ACACAAU
835	GGGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
845	GCGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
849	GUCUGCG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGG
872	CGCACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGC
883	GCAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACACGCA
885	CUGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGACACG
905	CGGUCGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGC
906	CCGGUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCG
919	GCUCACU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCC

936	GUACUGG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAU
937	GGUACUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCA
942	UGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAA
953	UCGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGC
962	CGGUGAC	CUGAUGAGGCCGAAAGGCCGAA	AUCGUCU
965	AUCCGGU	CUGAUGAGGCCGAAAGGCCGAA	ACGAUCG
973	UCUCCUC	CUGAUGAGGCCGAAAGGCCGAA	AUCCGGU
986	GUCCUUU	CUGAUGAGGCCGAAAGGCCGAA	ACGUUUC
996	GGUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
1005	GCUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
1006	UGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUCU
1028	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUC
1031	CCGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGACUC
1032	UCCGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGGACU
1033	GUCCGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAC
1058	CGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGG
1064	AUGCGUC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGA
1072	GCACAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGCGUC
1082	CUGCGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCACA
1083	GCUGCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCAC
1092	AGAAGCU	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCG
1097	GGGACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAG
1098	GGGGACA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGA
1102	GCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAG
1125	AAAGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGGCUG
1127	GUAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAGGGC
1131	UGACGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUA
1132	AUGACGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAU
1133	GAUGACG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGA
1137	CAGGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGUAAA
1140	GCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGACGU
1153	CAUAGUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGC
1158	CUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAU
1167	GGUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUCAUC
1168	UGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACUCAU
1169	AUGGUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACUCA
1182	AGAAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACACCAU
1183	CAGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AACACCA
1184	CCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAACACC
1187	UGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAAC
1188	CUGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAA
1198	CCUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGCC
1209	CAAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1215	CGGGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGA
1229	ACUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCC
1237	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGG
1250	GGGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGG
1268	AUGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGG

1279	GAGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
1281	CAGAGCU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
1286	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1309	GGACUGG CUGAUGAGGCCGAAAGGCCGAA ACAGGGG
1315	GGGCUAG CUGAUGAGGCCGAAAGGCCGAA ACUGGGA
1318	CUGGGGC CUGAUGAGGCCGAAAGGCCGAA AGGACUG
1331	GCCUGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCU
1334	ACAGCCU CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
1389	GGCCUCU CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
1413	AUCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUGCAG
1414	CAUCAUC CUGAUGAGGCCGAAAGGCCGAA AACUGCA
1437	GCCAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
1441	UGUUGCC CUGAUGAGGCCGAAAGGCCGAA AGCAAGG
1467	GUCUGUG CUGAUGAGGCCGAAAGGCCGAA ACACAGC
1468	GGUCUGU CUGAUGAGGCCGAAAGGCCGAA AACACAG
1482	GUCGACG CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
1486	AGUUGUC CUGAUGAGGCCGAAAGGCCGAA ACGGAUG
1494	AAACUCG CUGAUGAGGCCGAAAGGCCGAA AGUUGUC
1500	CUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACUCGGA
1501	GCUGCUG CUGAUGAGGCCGAAAGGCCGAA AACUCGG
1502	AGCUGCU CUGAUGAGGCCGAAAGGCCGAA AAACUCG
1525	CCACAGG CUGAUGAGGCCGAAAGGCCGAA AUGCCCU
1566	CUCAGGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAU
1577	CGAGUUA CUGAUGAGGCCGAAAGGCCGAA AGCCUCA
1579	GGCGAGU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
1583	ACUAGGC CUGAUGAGGCCGAAAGGCCGAA AGUUAUA
1588	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCGAG
1622	GGAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
1628	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
1648	CAUUGGG CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
1660	CUGAAAG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
1663	CUCCUGA CUGAUGAGGCCGAAAGGCCGAA AGGAGGC
1664	UCUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGGAGG
1665	AUCUCCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
1680	GGAGGAG CUGAUGAGGCCGAAAGGCCGAA AGUCUUC
1681	UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAGUCUU
1683	AAUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
1686	CGCAAUG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
1690	UGUCCGC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
1704	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AGUCCAU
1705	GGGCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUCCA
1707	CAGGGCU CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
1721	CUGAUCU CUGAUGAGGCCGAAAGGCCGAA ACUCAGC
1726	AGGAGCU CUGAUGAGGCCGAAAGGCCGAA AUCUGAC
1731	CCCUUAG CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1734	ACCCCCU CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
1754	CUCUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGCAG

Table 21
Human *rel A* Hairpin Ribozyme/Target Sequences

nt. Position	Hairpin Ribozyme sequence	Substrate
90	UGAGGGGG AGAA GUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACU GUU CCCCCUCA
156	GCUGCUUG AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCA GCC CAAGCAGC
362	GCCAUCCC AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGACU GCC GGGAUGGC
413	GUUCUGGA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACA GUU UCCAGAAC
606	GAAGGACA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCC GCC UGUCCUUC
652	UUGAGCUC AGAA GUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACACU GCC GAGCUCAA
695	CCCACCGA AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCU GCC UCGGUGGG
853	AGGCUGGG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCA GAC CCCAGCCU
900	GGUCGGAA AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGCG GCC UUCCGACC
955	UGACGAUC AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUACA GAC GAUCGUCA
1037	GUCGGUGG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCG GAC CCACCGAC
1045	GGCCGGGG AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACC GAC CCCCGGCC
1410	CAUCAUCA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCA GUU UGAUGAUG
1453	ACAGCUGG AGAA GUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCACA GAC CCAGCUGU
1471	GAUGCCAG AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACA GAC CUGGCAUC

Table 22

Mouse *rel A* Hairpin Ribozyme/Target Sequences

nt. Position	Hairpin Ribozyme sequence	Substrate
137	GUUGCUUC AGAA GUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACA GCC GAAGCAAC
273	GAGAUUCG AGAA GUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACA GUU CGAAUCUC
343	GCCAUCCC AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGACU GCC GGGAUGGC
366	GGGCAGAG AGAA GCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGCU GAC CUCUGCCC
633	UUGAGCUC AGAA GUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACACU GCC GAGCUCAA
676	CCCACCGA AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCU GCC UCGGUGGG
834	AGGCUGGG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCC GAC CCCAGCCU
881	GAUCAGAA AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGCG GCC UUCUGAUC
1100	AGGUGUAG AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGCA GCC CUACACCU
1205	GGGCAGAG AGAA GUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCACC GUC CUCUGCCC
1361	GGGCUUCC AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCU GUC GGAAGCCC
1385	CAGCAUCA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCA GUU UGAUGCUG
1431	ACUCCUGG AGAA GUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCACA GAC CCAGGAGU
1449	GAUGCCAG AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACA GAC CUGGCAUC
1802	AAGUCGGG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCU GCC CCCGACUU
2009	UGGCUCCA AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGACA GAC UGGAGCCA
2124	UGGUGUCG AGAA GCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGCU GCC CGACACCA
2233	AUUCUGAA AGAA GCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGCC GCC UUCAGAAU
2354	UCAGUAAA AGAA GUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGACA GCC UUUACUGA

Table 23: Human TNF-α HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
28	GGCAGGU U CUCUUCC		
29	GCAGGUU C UCUUCCU	321	GUCAGAU C AUCUUCU
31	AGGUUCU C UUCCUCU	324	AGAUCAU C UUCUCGA
33	GUUCUCU U CCUCUCA	326	AUCAUCU U CUCGAAC
34	UUCUCUU C CUCUCAC	327	UCAUCUU C UCGAACC
37	UCUUCCU C UCACAUA	329	AUCUUCU C GAACCCC
39	UUCCUCU C ACAUACU	352	AGCCUGU A GCCCAUG
44	CUCACAU A CUGACCC	361	CCCAUGU U GUAGCAA
58	CACGGCU C CACCCUC	364	AUGUUGU A GCAAACC
65	CCACCCU C UCUCCCC	374	AAACCCU C AAGCUGA
67	ACCCUCU C UCCCCUG	391	GGCAGCU C CAGUGGC
69	CCUCUCU C CCCUGGA	421	AUGCCCU C CUGGCCA
106	GCAUGAU C CGGGACG	449	GAGAGAU A ACCAGCU
136	AGGCGCU C CCCAAGA	468	GUGCCAU C AGAGGGC
165	CAGGGCU C CAGGCGG	480	GGCCUGU A CCUCAUC
177	CGGUGCU U GUUCCUC	484	UGUACCU C AUCUACU
180	UGCUUGU U CCUCAGC	487	ACCUCAU C UACUCCC
181	GCUUGUU C CUCAGCC	489	CUCAUCU A CUCCCAG
184	UGUUCCU C AGCCUCU	492	AUCUACU C CCAGGUC
190	UCAGCCU C UUCUCCU	499	CCCAGGU C CUCUUCA
192	AGCCUCU U CUCCUUC	502	AGGUCCU C UUCAAGG
193	GCCUCUU C UCCUUCC	504	GUCCUCU U CAAGGGC
195	CUCUUCU C CUUCCUG	505	UCCUCUU C AAGGGCC
198	UUCUCCU U CCUGAUC	525	UGCCCCU C CACCCAU
199	UCUCCUU C CUGAUCG	538	AUGUGCU C CUCACCC
205	UCCUGAU C GUGGCAG	541	UGCUCCU C ACCCACA
226	CCACGCU C UUCUGCC	553	ACACCAU C AGCCGCA
228	ACGCUCU U CUGCCUG	562	GCCGCAU C GCCGUCU
229	CGCUCUU C UGCCUGC	568	UCGCCGU C UCCUACC
243	CUGCACU U UGGAGUG	570	GCCGUCU C CUACCAG
244	UGCACUU U GGAGUGA	573	GUCUCCU A CCAGACC
253	GAGUGAU C GGCCCCC	586	CCAAGGU C AACCUCC
273	GAAGAGU C CCCCAGG	592	UCAACCU C CUCUCUG
286	GGGACCU C UCUCUAA	595	ACCUCCU C UCUGCCA
288	GACCUCU C UCUAAUC	597	CUCCUCU C UGCCAUC
290	CCUCUCU C UAAUCAG	604	CUGCCAU C AAGAGCC
292	UCUCUCU A AUCAGCC	657	CCCUGGU A UGAGCCC
295	CUCUAAU C AGCCCUC	667	AGCCCAU C UAUCUGG
302	CAGCCCU C UGGCCCA	669	CCCAUCU A UCUGGGA

671	CAUCUAU C UGGGAGG	960	UGGGAUU C AGGAAUG
682	GAGGGGU C UUCCAGC	1001	AACCACU A AGAAUUC
684	GGGGUCU U CCAGCUG	1007	UAAGAAU U CAAACUG
685	GGGUCUU C CAGCUGG	1008	AAGAAUU C AAACUGG
709	ACCGACU C AGCGCUG	1021	GGGGCCU C CAGAACU
721	CUGAGAU C AAUCGGC	1029	CAGAACU C ACUGGGG
725	GAUCAAU C GGCCCGA	1040	GGGGCCU A CAGCUUU
735	CCCGACU A UCUCGAC	1046	UACAGCU U UGAUCCC
737	CGACUAU C UCGACUU	1047	ACAGCUU U GAUCCCU
739	ACUAUCU C GACUUUG	1051	CUUUGAU C CCUGACA
744	CUCGACU U UGCCGAG	1060	CUGACAU C UGGAAUC
745	UCGACUU U GCCGAGU	1067	CUGGAAU C UGGAGAC
753	GCCGAGU C UGGGCAG	1085	GGAGCCU U UGGUUCU
763	GGCAGGU C UACUUUG	1086	GAGCCUU U GGUUCUG
765	CAGGUCU A CUUUGGG	1090	CUUUGGU U CUGGCCA
768	GUCUACU U UGGGAUC	1091	UUUGGUU C UGGCCAG
769	UCUACUU U GGGAUCA	1113	CAGGACU U GAGAAGA
775	UUGGGAU C AUUGCCC	1124	AAGACCU C ACCUAGA
778	GGAUCAU U GCCCUGU	1129	CUCACCU A GAAAUUG
801	CGAACAU C CAACCUU	1135	UAGAAAU U GACACAA
808	CCAACCU U CCCAAAC	1151	UGGACCU U AGGCCUU
809	CAACCUU C CCAAACG	1152	GGACCUU A GGCCUUC
820	AACGCCU C CCCUGCC	1158	UAGGCCU U CCUCUCU
833	CCCCAAU C CCUUUAU	1159	AGGCCUU C CUCUCUC
837	AAUCCCU U UAUUACC	1162	CCUUCCU C UCUCCAG
838	AUCCCUU U AUUACCC	1164	UUCCUCU C UCCAGAU
839	UCCCUUU A UUACCCC	1166	CCUCUCU C CAGAUGU
841	CCUUUAU U ACCCCCU	1174	CAGAUGU U UCCAGAC
842	CUUUAUU A CCCCCUC	1175	AGAUGUU U CCAGACU
849	ACCCCCU C CUUCAGA	1176	GAUGUUU C CAGACUU
852	CCCUCCU U CAGACAC	1183	CCAGACU U CCUUGAG
853	CCUCCUU C AGACACC	1184	CAGACUU C CUUGAGA
863	ACACCCU C AACCUCU	1187	ACUUCCU U GAGACAC
869	UCAACCU C UUCUGGC	1208	CAGCCCU C CCCAUGG
871	AACCUCU U CUGGCUC	1224	GCCAGCU C CCUCUAU
872	ACCUCUU C UGGCUCA	1228	GCUCCCU C UAUUUAU
878	UCUGGCU C AAAAAGA	1230	UCCCUCU A UUUAUGU
890	AGAGAAU U GGGGGCU	1232	CCUCUAU U UAUGUUU
898	GGGGGCU U AGGGUCG	1233	CCUCUAUU U AUGUUUG
899	GGGGCUU A GGGUCGG	1234	UCUAUUU A UGUUUGC
904	UUAGGGU C GGAACCC	1238	UUUAUGU U UGCACUU
917	CCAAGCU U AGAACUU	1239	UUAUGUU U GCACUUG
918	CAAGCUU A GAACUUU	1245	UUGCACU U GUGAUUA
924	UAGAACU U UAAGCAA	1251	UUGUGAU U AUUUAUU
925	AGAACUU U AAGCAAC	1252	UGUGAUU A UUUAUUA
926	GAACUUU A AGCAACA	1254	UGAUUAU U UAUUAUU
945	CACCACU U CGAAACC	1255	GAUUAUU U AUUAUUU
946	ACCACUU C GAAACCU	1256	AUUAUUU A UUAUUUA
959	CUGGGAU U CAGGAAU	1258	UAUUUAU U AUUUAUU

1259	AUUUAUU A UUUAUUU
1261	UUAUUAU U UAUUUAU
1262	UAUUAUU U AUUUAUU
1263	AUUAUUU A UUUAUUA
1265	UAUUUAU U UAUUAUU
1266	AUUUAUU U AUUAUUU
1267	UUUAUUU A UUAUUUA
1269	UAUUUAU U AUUUAUU
1270	AUUUAUU A UUUAUUU
1272	UUAUUAU U UAUUUAU
1273	UAUUAUU U AUUUAUU
1274	AUUAUUU A UUUAUUU
1276	UAUUUAU U UAUUUAC
1277	AUUUAUU U AUUUACA
1278	UUUAUUU A UUUACAG
1280	UAUUUAU U UACAGAU
1281	AUUUAUU U ACAGAUG
1282	UUUAUUU A CAGAUGA
1294	UGAAUGU A UUUAUUU
1296	AAUGUAU U UAUUUGG
1297	AUGUAUU U AUUUGGG
1298	UGUAUUU A UUUGGGA
1300	UAUUUAU U UGGGAGA
1301	AUUUAUU U GGGAGAC
1315	CCGGGGU A UCCUGGG
1317	GGGGUAU C CUGGGGG
1334	CCAAUGU A GGAGCUG
1345	GCUGCCU U GGCUCAG
1350	CUUGGCU C AGACAUG
1359	GACAUGU U UUCCGUG
1360	ACAUGUU U UCCGUGA
1361	CAUGUUU U CCGUGAA
1362	AUGUUUU C CGUGAAA
1386	GAACAAU A GGCUGUU
1393	AGGCUGU U CCCAUGU
1394	GGCUGUU C CCAUGUA
1401	CCCAUGU A GCCCCCU
1414	CUGGCCU C UGUGCCU
1422	UGUGCCU U CUUUUGA
1423	GUGCCUU C UUUUGAU
1425	GCCUUCU U UUGAUUA
1426	CCUUCUU U UGAUUAU
1427	CUUCUUU U GAUUAUG
1431	UUUUGAU U AUGUUUU
1432	UUUGAUU A UGUUUUU
1436	AUUAUGU U UUUUAAA
1437	UUAUGUU U UUUAAAA
1438	UAUGUUU U UUAAAAU
1440	UGUUUUU U AAAAUAU
1441	GUUUUUU A AAAUAUU
1446	UUAAAAU A UUAUCUG
1448	AAAAUAU U AUCUGAU
1449	AAAUAUU A UCUGAUU
1451	AUAUUAU C UGAUUAA
1456	AUCUGAU U AAGUUGU
1457	UCUGAUU A AGUUGUC
1461	AUUAAGU U GUCUAAA
1464	AAGUUGU C UAAACAA
1466	GUUGUCU A AACAAUG
1479	UGCUGAU U UGGUGAC
1480	GCUGAUU U GGUGACC
1494	CAACUGU C ACUCAUU
1498	UGUCACU C AUUGCUG
1501	CACUCAU U GCUGAGG
1512	GAGGCCU C UGCUCCC
1517	CUCUGCU C CCCAGGG
1528	AGGGAGU U GUGCCUG
1533	GUUGUGU C UGUAAUC
1537	UGUCUGU A AUCGGCC
1540	CUGUAAU C GGCCUAC
1546	UCGGCCU A CUAUUCA
1549	GCCUACU A UUCAGUG
1551	CUACUAU U CAGUGGC
1552	UACUAUU C AGUGGCG
1566	GAGAAAU A AAGGUUG
1572	UAAAGGU U GCUUAGG
1576	GGUUGCU U AGGAAAG
1577	GUUGCUU A GGAAAGA

Table 24: Human TNF-α Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
28	GGAAGAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
29	AGGAAGA CUGAUGAGGCCGAAAGGCCGAA AACCUGC
31	AGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAACCU
33	UGAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAAC
34	GUGAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
37	UAUGUGA CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
39	AGUAUGU CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
44	GGGUCAG CUGAUGAGGCCGAAAGGCCGAA AUGUGAG
58	GAGGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGUG
65	GGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGGUGG
67	CAGGGGA CUGAUGAGGCCGAAAGGCCGAA AGAGGGU
69	UCCAGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
106	CGUCCCG CUGAUGAGGCCGAAAGGCCGAA AUCAUGC
136	UCUUGGG CUGAUGAGGCCGAAAGGCCGAA AGCGCCU
165	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCCUG
177	GAGGAAC CUGAUGAGGCCGAAAGGCCGAA AGCACCG
180	GCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACAAGCA
181	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AACAAGC
184	AGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGAACA
190	AGGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
192	GAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
193	GGAAGGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
195	CAGGAAG CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
198	GAUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
199	CGAUCAG CUGAUGAGGCCGAAAGGCCGAA AAGGAGA
205	CUGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAGGA
226	GGCAGAA CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
228	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGAGCGU
229	GCAGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAGCG
243	CACUCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAG
244	UCACUCC CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
253	GGGGGCC CUGAUGAGGCCGAAAGGCCGAA AUCACUC
273	CCUGGGG CUGAUGAGGCCGAAAGGCCGAA ACUCUUC
286	UUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUCCC
288	GAUUAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
290	CUGAUUA CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
292	GGCUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
295	GAGGGCU CUGAUGAGGCCGAAAGGCCGAA AUUAGAG
302	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGGGCUG

321	AGAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAC
324	UCGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCU
326	GUUCGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAU
327	GGUUCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
329	GGGGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
352	CAUGGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
361	UUGCUAC	CUGAUGAGGCCGAAAGGCCGAA	ACAUGGG
364	GGUUUGC	CUGAUGAGGCCGAAAGGCCGAA	ACAACAU
374	UCAGCUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUUU
391	GCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCC
421	UGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCAU
449	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUC
468	GCCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAC
480	GAUGAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
484	AGUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGUACA
487	GGGAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGU
489	CUGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAG
492	GACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAU
499	UGAAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGGG
502	CCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGACCU
504	GCCCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAC
505	GGCCCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGA
525	AUGGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGCA
538	GGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACAU
541	UGUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
553	UGCGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
562	AGACGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGCGGC
568	GGUAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACGGCGA
570	CUGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AGACGGC
573	GGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAC
586	GGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACCUUGG
592	CAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
595	UGGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGU
597	GAUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAG
604	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAG
657	GGGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
667	CCAGAUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCU
669	UCCCAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGG
671	CCUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAUG
682	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCUC
684	CAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGACCCC
685	CCAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCC
709	CAGCGCU	CUGAUGAGGCCGAAAGGCCGAA	AGUCGGU
721	GCCGAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAG
725	UCGGGCC	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUC
735	GUCGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCGGG
737	AAGUCGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCG
739	CAAAGUC	CUGAUGAGGCCGAAAGGCCGAA	AGAUAGU
744	CUCGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCGAG

745	ACUCGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCGA
753	CUGCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGC
763	CAAAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGCC
765	CCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCUG
768	GAUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
769	UGAUCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUAGA
775	GGGCAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCAA
778	ACAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCC
801	AAGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUCG
808	GUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGG
809	CGUUUGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUUG
820	GGCAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUU
833	AUAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGGG
837	GGUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUU
838	GGGUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAU
839	GGGGUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGA
841	AGGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGG
842	GAGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAG
849	UCUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGU
852	GUGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGG
853	GGUGUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGAGG
863	AGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUGU
869	GCCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
871	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
872	UGAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGU
878	UCUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGA
890	AGCCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCU
898	CGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCC
899	CCGACCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCC
904	GGGUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUAA
917	AAGUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGG
918	AAAGUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUG
924	UUGCUUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUA
925	GUUGCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUCU
926	UGUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGUUC
945	GGUUUCG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGUG
946	AGGUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGU
959	AUUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCCAG
960	CAUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AAUCCCA
1001	GAAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGGUU
1007	CAGUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUA
1008	CCAGUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUU
1021	AGUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1029	CCCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUCUG
1040	AAAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCC
1046	GGGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUA
1047	AGGGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
1051	UGUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAG
1060	GAUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCAG

1067	GUCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAG
1085	AGAACCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCC
1086	CAGAACC	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUC
1090	UGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAG
1091	CUGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AACCAAA
1113	UCUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUG
1124	UCUAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUU
1129	CAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAG
1135	UUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUA
1151	AAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1152	GAAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCC
1158	AGAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUA
1159	GAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
1162	CUGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
1164	AUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAA
1166	ACAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGG
1174	GUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCUG
1175	AGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAUCU
1176	AAGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAACAUC
1183	CUCAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGG
1184	UCUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUG
1187	GUGUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGU
1208	CCAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCUG
1224	AUAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
1228	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGC
1230	ACAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
1232	AAACAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1233	CAAACAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAG
1234	GCAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGA
1238	AAGUGCA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAA
1239	CAAGUGC	CUGAUGAGGCCGAAAGGCCGAA	AACAUAA
1245	UAAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAA
1251	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
1252	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCACA
1254	AAUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUCA
1255	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUC
1256	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1258	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1259	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1261	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
1262	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUA
1263	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1265	AAUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1266	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1267	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1269	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1270	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1272	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
1273	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUA

1274	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1276	GUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1277	UGUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1278	CUGUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1280	AUCUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1281	CAUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1282	UCAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1294	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1296	CCAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUU
1297	CCCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
1298	UCCCAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
1300	UCUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1301	GUCUCCC	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1315	CCCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCGG
1317	CCCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUACCCC
1334	CAGCUCC	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
1345	CUGAGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
1350	CAUGUCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAG
1359	CACGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUC
1360	UCACGGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1361	UUCACGG	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1362	UUUCACG	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1386	AACAGCC	CUGAUGAGGCCGAAAGGCCGAA	AUUGUUC
1393	ACAUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCU
1394	UACAUGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCC
1401	AGGGGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAUGGG
1414	AGGCACA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
1422	UCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCACA
1423	AUCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGGCAC
1425	UAAUCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGGC
1426	AUAAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGG
1427	CAUAAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
1431	AAAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
1432	AAAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAUCAAA
1436	UUUAAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAU
1437	UUUUAAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUAA
1438	AUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUA
1439	UAUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1440	AUAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1441	AAUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAAC
1446	CAGAUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAA
1448	AUCAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUU
1449	AAUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1451	UUAAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAU
1456	ACAACUU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGAU
1457	GACAACU	CUGAUGAGGCCGAAAGGCCGAA	AAUCAGA
1461	UUUAGAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUAAU
1464	UUGUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAACUU
1466	CAUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AGACAAC

1479	GUCACCA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCA
1480	GGUCACC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAGC
1494	AAUGAGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUG
1498	CAGCAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGACA
1501	CCUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGAGUG
1512	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUC
1517	CCCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1528	CAGACAC	CUGAUGAGGCCGAAAGGCCGAA	ACUCCCU
1533	GAUUACA	CUGAUGAGGCCGAAAGGCCGAA	ACACAAC
1537	GGCCGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAGACA
1540	GUAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AUUACAG
1546	UGAAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGA
1549	CACUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGGC
1551	GCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AUAGUAG
1552	CGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGUA
1566	CAACCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUC
1572	CCUAAGC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUUA
1576	CUUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCAACC
1577	UCUUUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAC

Table 25: Mouse TNF-a HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
66	UgGAAAU a GcucCcA	324	GgGUGAU C GGuCCCC
101	GGCAGGU U CUgUcCC	347	GAGAagU u cCCAaaU
101	GGCAGgU u CuGUccC	364	CCUCcCU C UcAUCAG
102	GCAGGUU C UgUcCCU	366	UCcCUCU c AUCAGuu
102	gCAGgUU c ugUCCCU	366	UcCCUCU C auCAGuU
106	GUUCUgU c CCUuUCA	369	CUCUcAU C AGuuCUa
110	UgUcCCU u UCACucA	376	CAGuuCU a UGGCCCA
111	gUCcCUU u CaCUCAC	390	AgACCCU C AcaCUcA
111	guCCCuU u CACuCAc	396	ucaCAcU C AGAUCAU
112	UcCCUuU C ACucACU	401	cUCAGAU C AUCUUCU
116	UuUCACU C AcUGgcc	404	AGAUCAU C UUCUCaA
137	GCCaCAU C uCCcUCc	406	AUCAUCU U CUCaAAa
139	caCAuCU C CCUCcAg	406	AUcAUcU U cUcaAAA
177	GCAUGAU C CGcGACG	407	UCAUCUU C UCaAAau
207	AGGCaCU C CCCcAaA	409	AUCUUCU C aAAauuC
228	GGGGCuU C CAGAACU	409	AuCuuCU c AaAAUUC
228	GGGGCuU c CAGaacU	409	aUcUUcU c AAAauUc
236	CAGaaCU C CAGGCGG	432	AGCCUGU A GCCCAcG
236	CAGaACU c cAGgcGg		
249	GGugCCU a UgUCUcA		
249	GGuGCCU a UGucUCa	444	AcGUcGU A GCAAACC
		501	AcGCCCU C CUGGCCA
261	UCAGCCU C UUCUCaU	560	gGgUUGU a CCUuguC
261	UCAgCCU C UUCUcau	560	GGguUGU A CCUugUC
263	AGCCUCU U CUCaUUC	564	UGUACCU u gUCUACU
263	AgCCUCU U CUcauUC	567	ACCUugU C UACUCCC
264	GCCUCUU C UCaUUCC	569	CUugUCU A CUCCCAG
264	gCCUCUU C UcauUCc	572	gUCUACU C CCAGGUu
266	CUCUUCU C aUUCCUG	572	GUCUaCU c CCAGguu
269	UUCUCaU U CCUGcUu	572	GuCUacU C CCAgGUu
270	UCUCaUU C CUGcUuG	579	CCCAGGU u CUCUUCA
276	UCCUGcU u GUGGCAG	580	CCAGguU c uCUUcAa
297	CCACGCU C UUCUGuC	580	CCaGGuU c UCuUcaa
299	ACGCUCU U CUGuCUa	582	AGGUUCU C UUCaagg
300	CGCUCUU C UGuCUaC	582	AGGUuCU C UUCAAGG
304	CUuCUgU c uAcUGaa	584	GUuCUCU U CAAGGGa
306	UcUGUcU a cUgAAcU	585	UuCUCUU C AAGGGaC
314	CUGaACU U cGGgGUG	608	CcCGaCU a CgugCUC
315	UGaACUU c GGgGUGA	615	aCgUGcU C CUCAcCC
315	uGaaCUU c GGGguGa	615	AcGUGCU C CUCACCC
324	gGGUGaU c GgUCCcC	618	UGCUCCU C ACCCACA

630	ACACCgU C AGCCGau
630	ACACCgU C AgCCgaU
638	agcCgAU u uGCUaUc
643	aUUUGcU a uCUcAuA
645	UuGCuaU C UCaUACC
647	GCuaUCU C aUACCAG
663	agAAaGU C AACCUCC
669	UCAACCU C CUCUCUG
669	UcAAccU c cUcUCUG
672	ACCUCCU C UCUGCCg
674	CUCCUCU C UGCCgUC
681	cUGCCgU C AagaGcC
681	CUGCCgU C AAGAGCC
681	CUGcCgU C aaGAgcC
734	CCCUGGU A UGAGCCC
734	CccUGGU a ugaGCCc
744	AGCCCAU a UAcCUGG
746	CCCAUaU A cCUGGGA
759	GAgGAGU C uuCCAGc
759	GAGGaGU C UUCCAGC
761	GGaGUCU U CCAGCUG
762	GaGUCUU C CAGCUGG
786	ACCaACU C AGCGCUG
798	CUGAGgU C AAUCuGC
802	GgUCAAU C uGCCCaA
812	CCCaAgU A cuUaGAC
816	AgUAcuU a GACUUUG
821	uUaGACU U UGCgGAG
822	UaGACUU U GCgGAGU
830	GCgGAGU C cGGGCAG
840	GGCAGGU C UACUUUG
842	CAGGUCU A CUUUGGa
842	CAGgucU a CUUugGA
842	cagGuCU a CUUUgGA
845	GUCUACU U UGGagUC
846	UCUACUU U GGagUCA
852	UUGGagU C AUUGCuC
855	GagUCAU U GCuCUGU
887	AUCCaUU c ucUACCC
891	AuucuCU a CCCaGCC
905	CCcCaCU C UgaCCCC
905	cCCCacU c UgACCCC
905	CcCCACU c uGAccCC
914	GAcCCcU U uacUCUG
915	ACCCCuU u acUCuGA
919	CUUUAcU c ugaCCcC
928	GACCcCU u UaUugUC
928	gAcCCCU U UAUUguC
932	CCUUUAU U guCuaCU
940	GuCUACU c cUCAGaG
943	UACUccU C AGaGcCc
972	UCUaaCU u AgAAAGg
972	ucUaaCU u AGAaAgG
973	CUaACuU A GAAAggG
984	AGgGgAU U auGGcuc
984	AGGGgaU U aUGgCUc
985	GGGGauU a uGGcUCa
997	UcAGAgU c CAAcucu
1010	CuguGCU c AGAgCUU
1017	cAGAgCU U UcAaCAA
1018	AGAgCUU U cAaCAAC
1019	GAgCUUU c AaCAACu
1073	UgGGCCU c ucAUgCA
1096	AAGgAcU C AAAugGG
1106	aUGGGcU U uccGAAU
1107	UGGGcUU u ccGAAUu
1108	GGgCuUU c cGaaUUC
1115	CcGAAuU C ACUGGaG
1133	CGAAugU C CAuuCcU
1164	gagUGgU c AgGUUGc
1180	UcUgUcU c agaAUGA
1203	aaGAuCU c AGGCCUU
1210	cAGGCCU U CCUacCU
1211	AGGCCUU C CUacCUu
1214	CCUUCCU a cCUuCAG
1218	CcuACcU u CaGACCu
1218	CCuaCCU U CAGACcu
1218	cCuACcU u cAgACCU
1218	CCUacCU u CAGAccU
1219	CuaCCUU C AGACcuu
1219	CuAcCUU c agACcUU
1226	CagACCU U uCCAgAC
1226	CAGAccU U UCCAGAC
1227	agACCUU u CCAgACu
1227	AGAccUU U CCAGACU
1228	GAccUUU C CAGACUc
1238	gACUCuU c cCUGAGG
1262	CAGCCuU C CuCAcaG
1283	CCCCccU C uaUUUAU
1283	cCcCCCU C UAUUUAU
1285	cCCCUCU A UUUAUaU
1287	CcuCUAU u UauAuUU
1287	CCUCUAU U UAUaUUU
1288	CUCUAUU U AUaUUUG
1289	UCUAUUU A UaUUUGC
1293	UUUAUaU U UGCACUU
1293	uUUaUaU u UGcAcUu
1294	UUAUaUU U GCACUUa

1300	UUGCACU U aUuAUUu
1303	CAcuUaU u AuUuAUU
1304	acUuAUU A UUUAUUA
1306	UuAUUAU U UAUUAUU
1307	uAUUAUU U AUUAUUU
1307	UaUUaUU U AuuAUuU
1308	AUUAUUU A UUAUUUA
1310	UauUuAU U AUUUAUU
1310	UAUUUAU U AUUUAUU
1310	UAUUUAU U AUUUAUU
1311	AUUUAUU A UUUAUUU
1311	AUUUAUU A UUUAUUU
1311	AuuUAUU A UuUauUU
1313	UUAUUAU U UAUUUAU
1313	UUAUUAU U UAUUUAU
1313	uUAUUAU u UauUUAu
1314	UAUUAUU U AUUUAUU
1314	UAUUAUU U AUUUAUU
1315	AUUAUUU A UUUAUUA
1317	UAUUUAU U UAUUAUU
1318	AUUUAUU U AUUAUUU
1319	UUUAUUU A UUAUUUA
1326	AUUAUUU A UUUAUUU
1328	UAUUUAU U UAUUUgC
1329	AUUUAUU U AUUUgCu
1330	UUUAUUU A UUUgCuu
1332	UAUUUAU U UgCuuAU
1333	AUUUAUU U gCuuAUG
1337	auUUGCU U AuGAAuG
1338	uUUGCUU A uGAAuGu
1346	UGAAUGU A UUUAUUU
1348	AAUGUAU U UAUUUGG
1349	AUGUAUU U AUUUGGa
1350	UGUAUUU A UUUGGaA
1352	uAUuUAU u UGGaAGG
1352	UAUUUAU U UGGaAGg
1353	AUUUAUU U GGaAGgC
1369	GGGGUgU C CUGGaGG
1398	gCUguCU U cAGACAg
1398	GCUGuCU U cagaCAG
1412	GACAUGU U UUCuGUG
1413	ACAUGUU U UCuGUGA
1414	CAUGUUU U CuGUGAA
1415	AUGUUUU C uGUGAAA
1415	AUGUUUU c UgugAaA
1438	gaGCUGU c CCCAccU
1451	CUGGCCU C UcUaCCU
1453	ggCCUCU C UaCCuUG
1462	aCCuUGU u GCCuCCU
1470	GccuCcU C UUUUGcU
1472	cuCcUCU U UUGcUUA
1473	uCcUCUU U UGcUUAU
1474	CcUCUUU U GcUUAUG
1478	UUUUGcU U AUGUUUa
1479	UUUGcUU a UGuuuAa
1479	UUUGcUU A UGUUUaa
1484	UUAUGUU U aaaAcAA
1498	AAAuauU U AUCUaAc
1511	AcccAaU U GUCUuAA
1514	cAaUUGU C UuAAuAA
1516	aUUGUCU u AAuAAcG
1529	CgcugAU u UGGuGAC
1529	cGCUGAU U UGGUGAC
1530	gCUGAUU u gGUgacC
1530	GCUGAUU U GGUGACC
1563	UgaAcCU c UGcUCCC
1563	ugaaCCU C UGCUCCC
1568	CUCUGCU C CCCAcGG
1589	UGaCUGU A AUuGcCC
1592	CUGUAAU u GcCCUAC
1617	GAGAAAU A AAGaUcG
1623	UAAAGaU c GCUUAaa
1633	UUAaaaU a aaAAaCC
25	AgGgaCU a gCCagGA

Table 26: Mouse TNF-α Hammerhead Ribozyme Sequences

nt. Position	Mouse HH Ribozyme Sequence
25	UCCUGGC CUGAUGAGGCCGAAAGGCCGAA AGUCCCU
66	UGGGAGC CUGAUGAGGCCGAAAGGCCGAA AUUUCCA
101	GGGACAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
101	GGGACAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
102	AGGGACA CUGAUGAGGCCGAAAGGCCGAA AACCUGC
102	AGGGACA CUGAUGAGGCCGAAAGGCCGAA AACCUGC
106	UGAAAGG CUGAUGAGGCCGAAAGGCCGAA ACAGAAC
110	UGAGUGA CUGAUGAGGCCGAAAGGCCGAA AGGGACA
111	GUGAGUG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
111	GUGAGUG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
112	AGUGAGU CUGAUGAGGCCGAAAGGCCGAA AAAGGGA
115	GGCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUGAAA
137	GGAGGGA CUGAUGAGGCCGAAAGGCCGAA AUGUGGC
139	CUGGAGG CUGAUGAGGCCGAAAGGCCGAA AGAUGUG
177	CGUCGCG CUGAUGAGGCCGAAAGGCCGAA AUCAUGC
207	UUUGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGCCU
228	AGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
228	AGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
236	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
236	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
249	UGAGACA CUGAUGAGGCCGAAAGGCCGAA AGGCACC
249	UGAGACA CUGAUGAGGCCGAAAGGCCGAA AGGCACC
261	AUGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
261	AUGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
263	GAAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
263	GAAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
264	GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
264	GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
266	CAGGAAU CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
269	AAGCAGG CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
270	CAAGCAG CUGAUGAGGCCGAAAGGCCGAA AAUGAGA
276	CUGCCAC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
297	GACAGAA CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
299	UAGACAG CUGAUGAGGCCGAAAGGCCGAA AGAGCGU
300	GUAGACA CUGAUGAGGCCGAAAGGCCGAA AAGAGCG
304	UUCAGUA CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
306	AGUUCAG CUGAUGAGGCCGAAAGGCCGAA AGACAGA
314	CACCCCG CUGAUGAGGCCGAAAGGCCGAA AGUUCAG
315	UCACCCC CUGAUGAGGCCGAAAGGCCGAA AAGUUCA

315	UCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUUCA
324	GGGGACC	CUGAUGAGGCCGAAAGGCCGAA	AUCACCC
324	GGGGACC	CUGAUGAGGCCGAAAGGCCGAA	AUCACCC
347	AUUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUC
364	CUGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAGG
366	AACUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
366	AACUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGA
369	UAGAACU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAG
376	UGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACUG
390	UGAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCU
396	AUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGA
401	AGAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAG
404	UUGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAUCU
406	UUUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAU
406	UUUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAU
407	AUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
409	GAAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
409	GAAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
409	GAAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
432	CGUGGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
444	GGUUUGC	CUGAUGAGGCCGAAAGGCCGAA	ACGACGU
501	UGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCGU
560	GACAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAACCC
560	GACAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAACCC
564	AGUAGAC	CUGAUGAGGCCGAAAGGCCGAA	AGGUACA
567	GGGAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGU
569	CUGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGACAAG
572	AACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
572	AACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
572	AACCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
579	UGAAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCUGGG
580	UUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AACCUGG
580	UUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AACCUGG
582	CCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCU
582	CCUUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCU
584	UCCCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAC
585	GUCCCUU	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAA
608	GAGCACG	CUGAUGAGGCCGAAAGGCCGAA	AGUCGGG
615	GGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACGU
615	GGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCACGU
618	UGUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
630	AUCGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACGGUGU
630	AUCGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACGGUGU
638	GAUAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCGGCU
643	UAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAU
645	GGUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCAA
647	CUGGUAU	CUGAUGAGGCCGAAAGGCCGAA	AGAUAGC

663	GGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	ACUUUCU
669	CAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
669	CAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
672	CGGCAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGU
674	GACGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAG
681	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAG
681	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAG
681	GGCUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGGCAG
734	GGGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
734	GGGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
744	CCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCU
746	UCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGG
759	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCUC
759	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCUC
761	CAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGACUCC
762	CCAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACUC
786	CAGCGCU	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGU
798	GCAGAUU	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAG
802	UUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUUGACC
812	GUCUAAG	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGG
816	CAAAGUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUACU
821	CUCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUAA
822	ACUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUA
830	CUGCCCG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGC
840	CAAAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGCC
842	UCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCUG
842	UCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCUG
842	UCCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCUG
845	GACUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
846	UGACUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUAGA
852	GAGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAA
855	ACAGAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGACUC
887	GGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGGAU
891	GGCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAU
905	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
905	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
905	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
914	CAGAGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
915	UCAGAGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGGU
919	GGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUAAAG
928	GACAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
928	GACAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUC
932	AGUAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGG
940	CUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
943	GGGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGUA
972	CCUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAGA
972	CCUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAGA
973	CCCUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAG
984	GAGCCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCU

984	GAGCCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCU
985	UGAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUCCCC
997	AGAGUUG	CUGAUGAGGCCGAAAGGCCGAA	ACUCUGA
1010	AAGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
1017	UUGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCUG
1018	GUUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCUCU
1019	AGUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGCUC
1073	UGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
1096	CCCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUU
1106	AUUCGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCCAU
1107	AAUUCGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCCA
1108	GAAUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCC
1115	CUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCGG
1133	AGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCG
1164	GCAACCU	CUGAUGAGGCCGAAAGGCCGAA	ACCACUC
1180	UCAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGACAGA
1203	AAGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCUU
1210	AGGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1211	AAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCCU
1214	CUGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1218	AGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGG
1219	AAGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAG
1219	AAGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAG
1226	GUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
1226	GUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
1227	AGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1227	AGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1228	GAGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUC
1238	CCUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUC
1262	CUGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUG
1283	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGG
1283	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGG
1285	AUAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGG
1287	AAAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1287	AAAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1288	CAAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAG
1289	GCAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGA
1293	AAGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAA
1293	AAGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAA
1294	UAAGUGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAUAA
1300	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGCAA
1303	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGUG
1304	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAGU
1306	AAUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
1307	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUA
1307	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUA

1308	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1310	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1310	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1310	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1311	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1313	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
1313	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
1313	AUAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
1314	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUA
1314	AAUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUA
1315	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1317	AAUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1318	AAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1319	UAAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1326	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1328	GCAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1329	AGCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1330	AAGCAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1332	AUAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1333	CAUAAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1337	CAUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAU
1338	ACAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAA
1346	AAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1348	CCAAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUU
1349	UCCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
1350	UUCCAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
1352	CCUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1352	CCUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
1353	GCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1369	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	ACACCCC
1398	CUGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
1398	CUGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
1412	CACAGAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUC
1413	UCACAGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1414	UUCACAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1415	UUUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1415	UUUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1438	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUC
1451	AGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
1453	CAAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCC
1455	AACAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGG
1462	AGGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGU
1470	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGC
1472	UAAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAG
1473	AUAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGA
1474	CAUAAGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGG
1478	UAAACAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA

1479	UUAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAA
1479	UUAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAA
1484	UUGUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAUAA
1498	GUUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1511	UUAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUUGGGU
1514	UUAUUAA	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
1516	CGUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AGACAAU
1529	GUCACCA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCG
1529	GUCACCA	CUGAUGAGGCCGAAAGGCCGAA	AUCAGCG
1530	GGUCACC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAGC
1530	GGUCACC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAGC
1563	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCA
1563	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUCA
1568	CCGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1589	GGGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAGUCA
1592	GUAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUUACAG
1617	CGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUC
1623	UUUAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUA
1633	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAA

Table 27: Human TNF-α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
46	AGCCGUGG AGAA GUAUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACAUACU GAC CCACGGCU
54	GAGGGUGG AGAA GUGGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCCACG GCU CCACCCUC
185	GGAGAAGA AGAA GAGGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUCCUCA GCC UCUUCUCC
201	CUGCCACG AGAA GGAAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCUUCCU GAU CGUGGCAG
230	GUGCAGCA AGAA GAAGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCUUCU GCC UGCUGCAC
234	CAAAGUGC AGAA GGCAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCUGCCU GCU GCACUUUG
254	CCUCUGGG AGAA GAUCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGAUCG GCC CCCAGAGG
296	GGCCAGAG AGAA GAUUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUAAUCA GCC CUCUGGCC
317	AGAAGAUG AGAA GACUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCAGUCA GAU CAUCUUCU
387	GCCACUGG AGAA GCCCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGGGCA GCU CCAGUGGC
404	AUUGGCCC AGAA GUUCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGAACC GCC GGGCCAAU
453	GCACCACC AGAA GGUUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUAACCA GCU GGUGGUGC
518	GGUGGAGG AGAA GCCUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAAGGCU GCC CCUCCACC
554	GGCGAUGC AGAA GAUGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCAUCA GCC GCAUCGCC
565	UGGUAGGA AGAA GCGAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAUCGCC GUC UCCUACCA
576	UGACCUUG AGAA GGUAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCUACCA GAC CAAGGUCA
687	CCUUCUCC AGAA GGAAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCUUCCA GCU GGAGAAGG
704	AGCGCUGA AGAA GUCACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGUGACC GAC UCAGCGCU
726	GAUAGUCG AGAA GAUUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCAAUCG GCC CGACUAUC
730	UCGAGAUA AGAA GGCCGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCGGCCC GAC UAUCUCGA
824	GGGAUUGG AGAA GGGGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCCCCU GCC CCAAUCCC
1042	GGGAUCAA AGAA GUAGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCUACA GCU UUGAUCCC
1168	CUGGAAAC AGAA GGAGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCUCCA GAU GUUUCCAG
1178	UCAAGGAA AGAA GGAAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUUUCCA GAC UUCCUUGA
1202	AUGGGGAG AGAA GGGCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCCCA GCC CUCCCCAU
1220	AUAGAGGG AGAA GGCUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGAGCCA GCU CCCUCUAU
1284	AUACAUUC AGAA GUAAAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUUUACA GAU GAAUGUAU
1340	UGAGCCAA AGAA GCUCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGAGCU GCC UUGGCUCA
1390	UACAUGGG AGAA GCCUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUAGGCU GUU CCCAUGUA

1452	ACAACUUA	AGAA	GAUAAU	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUUAUCU	GAU	UAAGUUGU
1475	GUCACCAA	AGAA	GCAUUG	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAAUGCU	GAU	UUGGUGAC
1513	CCCUGGGG	AGAA	GAGGCC	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGCCUCU	GCU	CCCCAGGG
1541	GAAUAGUA	AGAA	GAUUAC	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUAAUCG	GCC	UACUAUUC

Table 28: Mouse TNF-α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
103	GUGAAAGG AGAA GAACCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGUUCU GUC CCUUUCAC
256	UGAGAAGA AGAA GAGACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGUCUCA GCC UCUUCUCA
272	CUGCCACA AGAA GGAAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAUUCCU GCU UGUGGCAG
301	GUUCAGUA AGAA GAAGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCUUCU GUC UACUGAAC
325	CCUUUGGG AGAA GAUCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGAUCG GUC CCCAAAGG
370	GGCCAUAG AGAA GAUGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCAUCA GUU CUAUGGCC
383	GUGUGAGG AGAA GGGCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGCCCA GAC CCUCACAC
397	AGAAGAUG AGAA GAGUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACACUCA GAU CAUCUUCU
467	GCCACUCC AGAA GCUCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGAGCA GCU GGAGUGGC
546	AACCCAUC AGAA GGCACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGUGCCA GCC GAUGGGUU
549	UACAACCC AGAA GCUGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCAGCC GAU GGGUUGUA
598	GUAGUCGG AGAA GCCUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAAGGCU GCC CCGACUAC
603	AGCACGUA AGAA GGGCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCCCC GAC UACGUGCU
631	AGCAAAUC AGAA GACGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCGUCA GCC GAUUUGCU
634	GAUAGCAA AGAA GCUGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUCAGCC GAU UUGCUAUC
675	CUCUUGAC AGAA GAGAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCUCUCU GCC GUCAAGAG
691	GUCCUUGG AGAA GGGGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGCCCCU GCC CCAAGGAC
764	CCUUCUCC AGAA GGAAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCUUCCA GCU GGAGAAGG
803	AGUACUUG AGAA GAUUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCAAUCU GCC CAAGUACU
895	AGAGUGGG AGAA GGGUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUACCCA GCC CCCACUCU
906	GUAAAGGG AGAA GAGUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACUCU GAC CCCUUUAC
920	AUAAAGGG AGAA GAGUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUACUCU GAC CCCUUUAU
953	AGGACACA AGAA GGGGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCCCCA GUC UGUGUCCU
1175	CAUUCUGA AGAA GAGGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGCCUCU GUC UCAGAAUG
1220	CUGGAAAG AGAA GAAGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCUUCA GAC CUUUCCAG
1230	AGGGAAGA AGAA GGAAAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUUUCCA GAC UCUUCCCU
1256	GUGAGGAA AGAA GUGCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUGCACA GCC UUCCUCAC
1274	UAGAGGGG AGAA GGCUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGAGCCA GCC CCCCUCUA

1393	UGUCUGAA	AGAA	GCUUCC	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGAAGCU	GUC	UUCAGACA
1435	CAGGUGGG	AGAA	GCUCAG	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGAGCU	GUC	CCCACCUG
1525	GUCACCAA	AGAA	GCGUUA	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAACGCU	GAU	UUGGUGAC
1542	GAUGUAGC	AGAA	GCCUGG	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCAGGCU	GUC	GCUACAUC
1564	CCGUGGGG	AGAA	GAGGUU	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AACCUCU	GCU	CCCCACGG

Table 29: Human *bcr/abl* HH Target Sequence

Sequence ID N .	HH Target Sequence
b2-a2 Junction	
20	UGACCAUCA AUA AGGAAGAAGCC
21	GAAGAAGCC CUU CAGCGGCCAGU
22	AAGAAGCCC UUC AGCGGCCAGUA
b3-a2 Junction	
23	UAAGCAGAG UUC AAAAGCCCUUC
24	UCAAAAGCC CUU CAGCGGCCAGU
25	CAAAAGCCC UUC AGCGGCCAGUA

Table 30: Human *bcr-abl* HH Ribozyme Sequences

Sequence ID No.	HH Ribozyme Sequence
26	GGCUUCUUCCU CUGAUGAGGCCGAAAGGCCGAA AUUGAUGGUCA
27	ACUGGCCGCUG CUGAUGAGGCCGAAAGGCCGAA AGGGCUUCUUC
28	UACUGGCCGCU CUGAUGAGGCCGAAAGGCCGAA AAGGGCUUCUU
29	GAAGGGCUUUU CUGAUGAGGCCGAAAGGCCGAA AACUCUGCUUA
30	ACUGGCCGCUG CUGAUGAGGCCGAAAGGCCGAA AGGGCUUUUGA
31	UACUGGCCGCU CUGAUGAGGCCGAAAGGCCGAA AAGGGCUUUUG

Table 31: RSV (1B) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
10	GGCAAAU A AAUCAAU	276	AAAAUAU A CUGAAUA
14	AAUAAAU C AAUUCAG	283	ACUGAAU A CAACACA
18	AAUCAAU U CAGCCAA	295	ACAAAAU A UGGCACU
19	AUCAAUU C AGCCAAC	303	UGGCACU U UCCCUAU
54	CAAUGAU A AUACACC	304	GGCACUU U CCCUAUG
57	UGAUAAU A CACCACA	305	GCACUUU C CCUAUGC
77	UGAUGAU C ACAGACA	309	UUUCCCU A UGCCAAU
94	AGACCGU U GUCACUU	317	UGCCAAU A UUCAUCA
97	CCGUUGU C ACUUGAG	319	CCAAUAU U CAUCAAU
101	UGUCACU U GAGACCA	320	CAAUAUU C AUCAAUC
110	AGACCAU A AUAACAU	323	UAUUCAU C AAUCAUG
113	CCAUAAU A ACAUCAC	327	CAUCAAU C AUGAUGG
118	AUAACAU C ACUAACC	337	GAUGGGU U CUUAGAA
122	CAUCACU A ACCAGAG	338	AUGGGUU C UUAGAAU
134	GAGACAU C AUAACAC	340	GGGUUCU U AGAAUGC
137	ACAUCAU A ACACACA	341	GGUUCUU A GAAUGCA
148	CACAAAU U UAUAUAC	350	AAUGCAU U GGCAUUA
149	ACAAAUU U AUAUACU	356	UUGGCAU U AAGCCUA
150	CAAAUUU A UAUACUU	357	UGGCAUU A AGCCUAC
152	AAUUUAU A UACUUGA	363	UAAGCCU A CAAAGCA
154	UUUAUAU A CUUGAUA	372	AAAGCAU A CUCCCAU
157	AUAUACU U GAUAAAU	375	GCAUACU C CCAUAAU
161	ACUUGAU A AAUCAUG	380	CUCCCAU A AUAUACA
165	GAUAAAU C AUGAAUG	383	CCAUAAU A UACAAGU
176	AAUGCAU A GUGAGAA	385	AUAAUAU A CAAGUAU
188	GAAAACU U GAUGAAA	391	UACAAGU A UGAUCUC
208	GCCACAU U UACAUUC	396	GUAUGAU C UCAAUCC
209	CCACAUU U ACAUUCC	398	AUGAUCU C AAUCCAU
210	CACAUUU A CAUUCCU	402	UCUCAAU C CAUAAAU
214	UUUACAU U CCUGGUC	406	AAUCCAU A AAUUUCA
215	UUACAUU C CUGGUCA	410	CAUAAAU U UCAACAC
221	UCCUGGU C AACUAUG	411	AUAAAUU U CAACACA
226	GUCAACU A UGAAAUG	412	UAAAUUU C AACACAA
239	UGAAACU A UUACACA	421	ACACAAU A UUCACAC
241	AAACUAU U ACACAAA	423	ACAAUAU U CACACAA
242	AACUAUU A CACAAAG	424	CAAUAUU C ACACAAU
251	ACAAAGU A GGAAGCA	432	ACACAAU C UAAAACA
261	AAGCACU A AAUAUAA	434	ACAAUCU A AAACAAC
265	ACUAAAU A UAAAAAA	446	AACAACU C UAUGCAU
267	UAAAUAU A AAAAAUA	448	CAACUCU A UGCAUAA
274	AAAAAAU A UACUGAA	454	UAUGCAU A ACUAUAC

458	CAUAACU A UACUCCA
460	UAACUAU A CUCCAUA
463	CUAUACU C CAUAGUC
467	ACUCCAU A GUCCAGA
470	CCAUAGU C CAGAUGG
489	UGAAAAU U AUAGUAA
490	GAAAAUU A UAGUAAU
492	AAAUUAU A GUAAUUU
495	UUAUAGU A AUUUAAA

Table 32: RSV (1B) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AUUGAUU CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
14	CUGAAUU CUGAUGAGGCCGAAAGGCCGAA AUUUAUU
18	UUGGCUG CUGAUGAGGCCGAAAGGCCGAA AUUGAUU
19	GUUGGCU CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
54	GGUGUAU CUGAUGAGGCCGAAAGGCCGAA AUCAUUG
57	UGUGGUG CUGAUGAGGCCGAAAGGCCGAA AUUAUCA
77	UGUCUGU CUGAUGAGGCCGAAAGGCCGAA AUCAUCA
94	AAGUGAC CUGAUGAGGCCGAAAGGCCGAA ACGGUCU
97	CUCAAGU CUGAUGAGGCCGAAAGGCCGAA ACAACGG
101	UGGUCUC CUGAUGAGGCCGAAAGGCCGAA AGUGACA
110	AUGUUAU CUGAUGAGGCCGAAAGGCCGAA AUGGUCU
113	GUGAUGU CUGAUGAGGCCGAAAGGCCGAA AUUAUGG
118	GGUUAGU CUGAUGAGGCCGAAAGGCCGAA AUGUUAU
122	CUCUGGU CUGAUGAGGCCGAAAGGCCGAA AGUGAUG
134	GUGUUAU CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
137	UGUGUGU CUGAUGAGGCCGAAAGGCCGAA AUGAUGU
148	GUAUAUA CUGAUGAGGCCGAAAGGCCGAA AUUUGUG
149	AGUAUAU CUGAUGAGGCCGAAAGGCCGAA AAUUUGU
150	AAGUAUA CUGAUGAGGCCGAAAGGCCGAA AAAUUUG
152	UCAAGUA CUGAUGAGGCCGAAAGGCCGAA AUAAAUU
154	UAUCAAG CUGAUGAGGCCGAAAGGCCGAA AUAUAAA
157	AUUUAUC CUGAUGAGGCCGAAAGGCCGAA AGUAUAU
161	CAUGAUU CUGAUGAGGCCGAAAGGCCGAA AUCAAGU
165	CAUUCAU CUGAUGAGGCCGAAAGGCCGAA AUUUAUC
176	UUCUCAC CUGAUGAGGCCGAAAGGCCGAA AUGCAUU
188	UUUCAUC CUGAUGAGGCCGAAAGGCCGAA AGUUUUC
208	GAAUGUA CUGAUGAGGCCGAAAGGCCGAA AUGUGGC
209	GGAAUGU CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
210	AGGAAUG CUGAUGAGGCCGAAAGGCCGAA AAAUGUG
214	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AUGUAAA
215	UGACCAG CUGAUGAGGCCGAAAGGCCGAA AAUGUAA
221	CAUAGUU CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
226	CAUUUCA CUGAUGAGGCCGAAAGGCCGAA AGUUGAC
239	UGUGUAA CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
241	UUUGUGU CUGAUGAGGCCGAAAGGCCGAA AUAGUUU
242	CUUUGUG CUGAUGAGGCCGAAAGGCCGAA AAUAGUU
251	UGCUUCC CUGAUGAGGCCGAAAGGCCGAA ACUUUGU
261	UUAUAUU CUGAUGAGGCCGAAAGGCCGAA AGUGCUU
265	UUUUUUA CUGAUGAGGCCGAAAGGCCGAA AUUUAGU
267	UAUUUUU CUGAUGAGGCCGAAAGGCCGAA AUAUUUA
274	UUCAGUA CUGAUGAGGCCGAAAGGCCGAA AUUUUUU
276	UAUUCAG CUGAUGAGGCCGAAAGGCCGAA AUAUUUU

283	UGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGU
295	AGUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUUGU
303	AUAGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCCA
304	CAUAGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGCC
305	GCAUAGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGC
309	AUUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAA
317	UGAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCA
319	AUUGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUUGG
320	GAUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUG
323	CAUGAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGAAUA
327	CCAUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUG
337	UUCUAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCCAUC
338	AUUCUAA	CUGAUGAGGCCGAAAGGCCGAA	AACCCAU
340	GCAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAACCC
341	UGCAUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGAACC
350	UAAUGCC	CUGAUGAGGCCGAAAGGCCGAA	AUGCAUU
356	UAGGCUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAA
357	GUAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AAUGCCA
363	UGCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUA
372	AUGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
375	AUUAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAUGC
380	UGUAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAG
383	ACUUGUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUGG
385	AUACUUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAU
391	GAGAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGUA
396	GGAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUAC
398	AUGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAU
402	AUUUAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGA
406	UGAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUU
410	GUGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUG
411	UGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUUAU
412	UUGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUUA
421	GUGUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUGU
423	UUGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUAUUGU
424	AUUGUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUG
432	UGUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUGU
434	GUUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
446	AUGCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUU
448	UUAUGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
454	GUAUAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGCAUA
458	UGGAGUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUG
460	UAUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AUAGUUA
463	GACUAUG	CUGAUGAGGCCGAAAGGCCGAA	AGUAUAG
467	UCUGGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGU
470	CCAUCUG	CUGAUGAGGCCGAAAGGCCGAA	ACUAUGG
489	UUACUAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUUCA
490	AUUACUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUUUC
492	AAAUUAC	CUGAUGAGGCCGAAAGGCCGAA	AUAAUUU
495	UUUAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACUAUAA

Table 33 : RSV (1C) HH target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
10	GGCAAAU A AGAAUUU	165	UACAUUU A ACUAACG
16	UAAGAAU U UGAUAAG	169	UUUAACU A ACGCUUU
17	AAGAAUU U GAUAAGU	175	UAACGCU U UGGCUAA
21	AUUUGAU A AGUACCA	176	AACGCUU U GGCUAAG
25	GAUAAGU A CCACUUA	181	UUUGGCU A AGGCAGU
31	UACCACU U AAAUUUA	192	CAGUGAU A CAUACAA
32	ACCACUU A AAUUUAA	196	GAUACAU A CAAUCAA
36	CUUAAAU U UAACUCC	201	AUACAAU C AAAUUGA
37	UUAAAUU U AACUCCC	206	AUCAAAU U GAAUGGC
38.	UAAAUUU A ACUCCCU	216	AUGGCAU U GUGUUUG
42	UUUAACU C CCUUGGU	221	AUUGUGU U UGUGCAU
46	ACUCCCU U GGUUAGA	222	UUGUGUU U GUGCAUG
50	CCUUGGU U AGAGAUG	231	UGCAUGU U AUUACAA
51	CUUGGUU A GAGAUGG	232	GCAUGUU A UUACAAG
67	CAGCAAU U CAUUGAG	234	AUGUUAU U ACAAGUA
68	AGCAAUU C AUUGAGU	235	UGUUAUU A CAAGUAG
71	AAUUCAU U GAGUAUG	241	UACAAGU A GUGAUAU
76	AUUGAGU A UGAUAAA	247	UAGUGAU A UUUGCCC
81	GUAUGAU A AAAGUUA	249	GUGAUAU U UGCCCUA
87	UAAAAGU U AGAUUAC	250	UGAUAUU U GCCCUAA
88	AAAAGUU A GAUUACA	256	UUGCCCU A AUAAUAA
92	GUUAGAU U ACAAAAU	259	CCCUAAU A AUAAUAU
93	UUAGAUU A CAAAAUU	262	UAAUAAU A AUAUUGU
100	ACAAAAU U UGUUUGA	265	UAAUAAU A UUGUAGU
101	CAAAAUU U GUUUGAC	267	AUAAUAU U GUAGUAA
104	AAUUUGU U UGACAAU	270	AUAUUGU A GUAAAAU
105	AUUUGUU U GACAAUG	273	UUGUAGU A AAAUCCA
120	AUGAAGU A GCAUUGU	278	GUAAAAU C CAAUUUC
125	GUAGCAU U GUUAAAA	283	AUCCAAU U UCACAAC
128	GCAUUGU U AAAAAUA	284	UCCAAUU U CACAACA
129	CAUUGUU A AAAAUAA	285	CCAAUUU C ACAACAA
135	UAAAAAU A ACAUGCU	300	UGCCAGU A CUACAAA
143	ACAUGCU A UACUGAU	303	CAGUACU A CAAAAUG
145	AUGCUAU A CUGAUAA	316	UGGAGGU U AUAUAUG
151	UACUGAU A AAUUAAU	317	GGAGGUU A UAUAUGG
155	GAUAAAU U AAUACAU	319	AGGUUAU A UAUGGGA
156	AUAAAUU A AUACAUU	321	GUUAUAU A UGGGAAA
159	AAUUAAU A CAUUUAA	338	AUGGAAU U AACACAU
163	AAUACAU U UAACUAA	339	UGGAAUU A ACACAUU
164	AUACAUU U AACUAAC	346	AACACAU U GCUCUCA

350	CAUUGCU	C	UCAACCU
352	UUGCUCU	C	AACCUAA
358	UCAACCU	A	AUGGUCU
364	UAAUGGU	C	UACUAGA
366	AUGGUCU	A	CUAGAUG
369	GUCUACU	A	GAUGACA
379	UGACAAU	U	GUGAAAU
387	GUGAAAU	U	AAAUUCU
388	UGAAAUU	A	AAUUCUC
392	AUUAAAU	U	CUCCAAA
393	UUAAAUU	C	UCCAAAA
395	AAAUUCU	C	CAAAAAA
405	AAAAACU	A	AGUGAUU
412	AAGUGAU	U	CAACAAU
413	AGUGAUU	C	AACAAUG
427	GACCAAU	U	AUAUGAA
428	ACCAAUU	A	UAUGAAU
430	CAAUUAU	A	UGAAUCA
436	UAUGAAU	C	AAUUAUC
440	AAUCAAU	U	AUCUGAA
441	AUCAAUU	A	UCUGAAU
443	CAAUUAU	C	UGAAUUA
449	UCUGAAU	U	ACUUGGA
450	CUGAAUU	A	CUUGGAU
453	AAUUACU	U	GGAUUUG
458	CUUGGAU	U	UGAUCUU
459	UUGGAUU	U	GAUCUUA
463	AUUUGAU	C	UUAAUCC
465	UUGAUCU	U	AAUCCAU
466	UGAUCUU	A	AUCCAUA
469	UCUUAAU	C	CAUAAAU
473	AAUCCAU	A	AAUUAUA
477	CAUAAAU	U	AUAAUUA
478	AUAAAUU	A	UAAUUAA
480	AAAUUAU	A	AUUAAUA
483	UUAUAAU	U	AAUAUCA
484	UAUAAUU	A	AUAUCAA
487	AAUUAAU	A	UCAACUA
489	UUAAUAU	C	AACUAGC
494	AUCAACU	A	GCAAAUC
501	AGCAAAU	C	AAUGUCA
507	UCAAUGU	C	ACUAACA
511	UGUCACU	A	ACACCAU
519	ACACCAU	U	AGUUAAU
520	CACCAUU	A	GUUAAUA
523	CAUUAGU	U	AAUAUAA
524	AUUAGUU	A	AUAUAAA

Table 34: RSV (1C) HH Ribozyme Sequence

nt. Positi n	HH Ribozyme Sequence
10	AAAUUCU CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
16	CUUAUCA CUGAUGAGGCCGAAAGGCCGAA AUUCUUA
17	ACUUAUC CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
21	UGGUACU CUGAUGAGGCCGAAAGGCCGAA AUCAAAU
25	UAAGUGG CUGAUGAGGCCGAAAGGCCGAA ACUUAUC
31	UAAAUUU CUGAUGAGGCCGAAAGGCCGAA AGUGGUA
32	UUAAAUU CUGAUGAGGCCGAAAGGCCGAA AAGUGGU
36	GGAGUUA CUGAUGAGGCCGAAAGGCCGAA AUUUAAG
37	GGGAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUUAA
38	AGGGAGU CUGAUGAGGCCGAAAGGCCGAA AAAUUUA
42	ACCAAGG CUGAUGAGGCCGAAAGGCCGAA AGUUAAA
46	UCUAACC CUGAUGAGGCCGAAAGGCCGAA AGGGAGU
50	CAUCUCU CUGAUGAGGCCGAAAGGCCGAA ACCAAGG
51	CCAUCUC CUGAUGAGGCCGAAAGGCCGAA AACCAAG
67	CUCAAUG CUGAUGAGGCCGAAAGGCCGAA AUUGCUG
68	ACUCAAU CUGAUGAGGCCGAAAGGCCGAA AAUUGCU
71	CAUACUC CUGAUGAGGCCGAAAGGCCGAA AUGAAUU
76	UUUAUCA CUGAUGAGGCCGAAAGGCCGAA ACUCAAU
81	UAACUUU CUGAUGAGGCCGAAAGGCCGAA AUCAUAC
87	GUAAUCU CUGAUGAGGCCGAAAGGCCGAA ACUUUUA
88	UGUAAUC CUGAUGAGGCCGAAAGGCCGAA AACUUUU
92	AUUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCUAAC
93	AAUUUUG CUGAUGAGGCCGAAAGGCCGAA AAUCUAA
100	UCAAACA CUGAUGAGGCCGAAAGGCCGAA AUUUUGU
101	GUCAAAC CUGAUGAGGCCGAAAGGCCGAA AAUUUUG
104	AUUGUCA CUGAUGAGGCCGAAAGGCCGAA ACAAAUU
105	CAUUGUC CUGAUGAGGCCGAAAGGCCGAA AACAAAU
120	ACAAUGC CUGAUGAGGCCGAAAGGCCGAA ACUUCAU
125	UUUUAAC CUGAUGAGGCCGAAAGGCCGAA AUGCUAC
128	UAUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAAUGC
129	UUAUUUU CUGAUGAGGCCGAAAGGCCGAA AACAAUG
135	AGCAUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
143	AUCAGUA CUGAUGAGGCCGAAAGGCCGAA AGCAUGU
145	UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AUAGCAU
151	AUUAAUU CUGAUGAGGCCGAAAGGCCGAA AUCAGUA
155	AUGUAUU CUGAUGAGGCCGAAAGGCCGAA AUUUAUC
156	AAUGUAU CUGAUGAGGCCGAAAGGCCGAA AAUUUAU
159	UUAAAUG CUGAUGAGGCCGAAAGGCCGAA AUUAAUU
163	UUAGUUA CUGAUGAGGCCGAAAGGCCGAA AUGUAUU
164	GUUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUGUAU
165	CGUUAGU CUGAUGAGGCCGAAAGGCCGAA AAAUGUA

169	AAAGCGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAAA
175	UUAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCGUUA
176	CUUAGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGCGUU
181	ACUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAA
192	UUGUAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACUG
196	UUGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGUAUC
201	UCAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUGUAU
206	GCCAUUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUGAU
216	CAAACAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAU
221	AUGCACA	CUGAUGAGGCCGAAAGGCCGAA	ACACAAU
222	CAUGCAC	CUGAUGAGGCCGAAAGGCCGAA	AACACAA
231	UUGUAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAUGCA
232	CUUGUAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGC
234	UACUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUAACAU
235	CUACUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUAACA
241	AUAUCAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUGUA
247	GGGCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCACUA
249	UAGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUCAC
250	UUAGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAUCA
256	UUAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGGGCAA
259	AUAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAGGG
262	ACAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUUA
265	ACUACAA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUUA
267	UUACUAC	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAU
270	AUUUUAC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUAU
273	UGGAUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUACAA
278	GAAAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUAC
283	GUUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAU
284	UGUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGA
285	UUGUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
300	UUUGUAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGCA
303	CAUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGUACUG
316	CAUAUAU	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
317	CCAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AACCUCC
319	UCCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAACCU
321	UUUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAAC
338	AUGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAU
339	AAUGUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCA
346	UGAGAGC	CUGAUGAGGCCGAAAGGCCGAA	AUGUGUU
350	AGGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAUG
352	UUAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGCAA
358	AGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
364	UCUAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUUA
366	CAUCUAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCAU
369	UGUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUAGAC
379	AUUUCAC	CUGAUGAGGCCGAAAGGCCGAA	AUUGUCA
387	AGAAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAC
388	GAGAAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
392	UUUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUAAU

393	UUUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUUUAA
395	UUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUU
405	AAUCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUU
412	AUUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACUU
413	CAUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUCACU
427	UUCAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUC
428	AUUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGU
430	UGAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUUG
436	GAUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAUA
440	UUCAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUU
441	AUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUUGAU
443	UAAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUUG
449	UCCAAGU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGA
450	AUCCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCAG
453	CAAAUCC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAUU
458	AAGAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAAG
459	UAAGAUC	CUGAUGAGGCCGAAAGGCCGAA	AAUCCAA
463	GGAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAU
465	AUGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAA
466	UAUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCA
469	AUUUAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGA
473	UAUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUU
477	UAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUG
478	UUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUUAU
480	UAUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUUU
483	UGAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAA
484	UUGAUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA
487	UAGUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAUU
489	GCUAGUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAA
494	GAUUUGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAU
501	UGACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUGCU
507	UGUUAGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGA
511	AUGGUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUGACA
519	AUUAACU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
520	UAUUAAC	CUGAUGAGGCCGAAAGGCCGAA	AAUGGUG
523	UUAUAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUAAUG
524	UUUAUAU	CUGAUGAGGCCGAAAGGCCGAA	AACUAAU

Table 35: RSV (N) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	GGCAAAU A CAAAGAU	217	GGUAUGU U AUAUGCG
21	GAUGGCU C UUAGCAA	218	GUAUGUU A UAUGCGA
23	UGGCUCU U AGCAAAG	220	AUGUUAU A UGCGAUG
24	GGCUCUU A GCAAAGU	229	GCGAUGU C UAGGUUA
32	GCAAAGU C AAGUUGA	231	GAUGUCU A GGUUAGG
37	GUCAAGU U GAAUGAU	235	UCUAGGU U AGGAAGA
45	GAAUGAU A CACUCAA	236	CUAGGUU A GGAAGAG
50	AUACACU C AACAAAG	254	ACACCAU A AAAAUAC
60	CAAAGAU C AACUUCU	260	UAAAAAU A CUCAGAG
65	AUCAACU U CUGUCAU	263	AAAUACU C AGAGAUG
66	UCAACUU C UGUCAUC	277	GCGGGAU A UCAUGUA
70	CUUCUGU C AUCCAGC	279	GGGAUAU C AUGUAAA
73	CUGUCAU C CAGCAAA	284	AUCAUGU A AAAGCAA
82	AGCAAAU A CACCAUC	299	AUGGAGU A GAUGUAA
89	ACACCAU C CAACGGA	305	UAGAUGU A ACAACAC
108	AGGAGAU A GUAUUGA	315	AACACAU C GUCAAGA
111	AGAUAGU A UUGAUAC	318	ACAUCGU C AAGACAU
113	AUAGUAU U GAUACUC	326	AAGACAU U AAUGGAA
117	UAUUGAU A CUCCUAA	327	AGACAUU A AUGGAAA
120	UGAUACU C CUAAUUA	346	AUGAAAU U UGAAGUG
123	UACUCCU A AUUAUGA	347	UGAAAUU U GAAGUGU
126	UCCUAAU U AUGAUGU	355	GAAGUGU U AACAUUG
127	CCUAAUU A UGAUGUG	356	AAGUGUU A ACAUUGG
146	AACACAU C AAUAAGU	361	UUAACAU U GGCAAGC
150	CAUCAAU A AGUUAUG	370	GCAAGCU U AACAACU
154	AAUAAGU U AUGUGGC	371	CAAGCUU A ACAACUG
155	AUAAGUU A UGUGGCA	383	CUGAAAU U CAAAUCA
166	GGCAUGU U AUUAAUC	384	UGAAAUU C AAAUCAA
167	GCAUGUU A UUAAUCA	389	UUCAAAU C AACAUUG
169	AUGUUAU U AAUCACA	395	UCAACAU U GAGAUAG
170	UGUUAUU A AUCACAG	401	UUGAGAU A GAAUCUA
173	UAUUAAU C ACAGAAG	406	AUAGAAU C UAGAAAA
186	AGAUGCU A AUCAUAA	408	AGAAUCU A GAAAAUC
189	UGCUAAU C AUAAAUU	415	AGAAAAU C CUACAAA
192	UAAUCAU A AAUUCAC	418	AAAUCCU A CAAAAAA
196	CAUAAAU U CACUGGG	431	AAAUGCU A AAAGAAA
197	AUAAAUU C ACUGGGU	449	GAGAGGU A GCUCCAG
205	ACUGGGU U AAUAGGU	453	GGUAGCU C CAGAAUA
206	CUGGGUU A AUAGGUA	460	CCAGAAU A CAGGCAU
209	GGUUAAU A GGUAUGU	472	CAUGACU C UCCUGAU
213	AAUAGGU A UGUUAUA	474	UGACUCU C CUGAUUG

480	UCCUGAU U GUGGGAU
491	GGAUGAU A AUAUUAU
494	UGAUAAU A UUAUGUA
496	AUAAUAU U AUGUAUA
497	UAAUAUU A UGUAUAG
501	AUUAUGU A UAGCAGC
503	UAUGUAU A GCAGCAU
511	GCAGCAU U AGUAAUA
512	CAGCAUU A GUAAUAA
515	CAUUAGU A AUAACUA
518	UAGUAAU A ACUAAAU
522	AAUAACU A AAUUAGC
526	ACUAAAU U AGCAGCA
527	CUAAAUU A GCAGCAG
544	GACAGAU C UGGUCUU
549	AUCUGGU C UUACAGC
551	CUGGUCU U ACAGCCG
552	UGGUCUU A CAGCCGU
563	CCGUGAU U AGGAGAG
564	CGUGAUU A GGAGAGC
573	GAGAGCU A AUAAUGU
576	AGCUAAU A AUGUCCU
581	AUAAUGU C CUAAAAA
584	AUGUCCU A AAAAAUG
603	GAAACGU U ACAAAGG
604	AAACGUU A CAAAGGC
613	AAAGGCU U ACUACCC
614	AAGGCUU A CUACCCA
617	GCUUACU A CCCAAGG
629	AGGACAU A GCCAACA
640	AACAGCU U CUAUGAA
641	ACAGCUU C UAUGAAG
643	AGCUUCU A UGAAGUG
652	GAAGUGU U UGAAAAA
653	AAGUGUU U GAAAAAC
663	AAAACAU C CCCACUU
670	CCCCACU U UAUAGAU
671	CCCACUU U AUAGAUG
672	CCACUUU A UAGAUGU
674	ACUUUAU A GAUGUUU
680	UAGAUGU U UUUGUUC
681	AGAUGUU U UUGUUCA
682	GAUGUUU U UGUUCAU
683	AUGUUUU U GUUCAUU
686	UUUUUGU U CAUUUUG
687	UUUUGUU C AUUUUGG
690	UGUUCAU U UUGGUAU
691	GUUCAUU U UGGUAUA
692	UUCAUUU U GGUAUAG
696	UUUUGGU A UAGCACA
698	UUGGUAU A GCACAAU
706	GCACAAU C UUCUACC
708	ACAAUCU U CUACCAG
709	CAAUCUU C UACCAGA
711	AUCUUCU A CCAGAGG
726	UGGCAGU A GAGUUGA
731	GUAGAGU U GAAGGGA
740	AAGGGAU U UUUGCAG
741	AGGGAUU U UUGCAGG
742	GGGAUUU U UGCAGGA
743	GGAUUUU U GCAGGAU
751	GCAGGAU U GUUUAUG
754	GGAUUGU U UAUGAAU
755	GAUUGUU U AUGAAUG
756	AUUGUUU A UGAAUGC
766	AAUGCCU A UGGUGCA
787	GUGAUGU U ACGGUGG
788	UGAUGUU A CGGUGGG
800	GGGGAGU C UUAGCAA
802	GGAGUCU U AGCAAAA
803	GAGUCUU A GCAAAAU
811	GCAAAAU C AGUUAAA
815	AAUCAGU U AAAAAUA
816	AUCAGUU A AAAAUAU
822	UAAAAAU A UUAUGUU
824	AAAAUAU U AUGUUAG
825	AAAUAUU A UGUUAGG
829	AUUAUGU U AGGACAU
830	UUAUGUU A GGACAUG
840	ACAUGCU A GUGUGCA
866	AACAAGU U GUUGAGG
869	AAGUUGU U GAGGUUU
875	UUGAGGU U UAUGAAU
876	UGAGGUU U AUGAAUA
877	GAGGUUU A UGAAUAU
883	UAUGAAU A UGCCCAA
895	CAAAAAU U GGGUGGU
913	GCAGGAU U CUACCAU
914	CAGGAUU C UACCAUA
916	GGAUUCU A CCAUAUA
921	CUACCAU A UAUUGAA
923	ACCAUAU A UUGAACA
925	CAUAUAU U GAACAAC
943	AAAGCAU C AUUAUUA
946	GCAUCAU U AUUAUCU
947	CAUCAUU A UUAUCUU
949	UCAUUAU U AUCUUUG
950	CAUUAUU A UCUUUGA

952	UUAUUAU C UUUGACU
954	AUUAUCU U UGACUCA
955	UUAUCUU U GACUCAA
960	UUUGACU C AAUUUCC
964	ACUCAAU U UCCUCAC
965	CUCAAUU U CCUCACU
966	UCAAUUU C CUCACUU
969	AUUUCCU C ACUUCUC
973	CCUCACU U CUCCAGU
974	CUCACUU C UCCAGUG
976	CACUUCU C CAGUGUA
983	CCAGUGU A GUAUUAG
986	GUGUAGU A UUAGGCA
988	GUAGUAU U AGGCAAU
989	UAGUAUU A GGCAAUG
1007	CUGGCCU A GGCAUAA
1013	UAGGCAU A AUGGGAG
1024	GGAGAGU A CAGAGGU
1032	CAGAGGU A CACCGAG
1044	GAGGAAU C AAGAUCU
1050	UCAAGAU C UAUAUGA
1052	AAGAUCU A UAUGAUG
1054	GAUCUAU A UGAUGCA
1072	AAGGCAU A UGCUGAA
1085	AACAACU C AAAGAAA
1103	GUGUGAU U AACUACA
1104	UGUGAUU A ACUACAG
1108	AUUAACU A CAGUGUA
1115	ACAGUGU A CUAGACU
1118	GUGUACU A GACUUGA
1123	CUAGACU U GACAGCA
1139	AAGAACU A GAGGCUA
1146	AGAGGCU A UCAAACA
1148	AGGCUAU C AAACAUC
1155	CAAACAU C AGCUUAA
1160	AUCAGCU U AAUCCAA
1161	UCAGCUU A AUCCAAA
1164	GCUUAAU C CAAAAGA
1173	AAAAGAU A AUGAUGU
1181	AUGAUGU A GAGCUUU
1187	UAGAGCU U UGAGUUA
1188	AGAGCUU U GAGUUAA
1193	UUUGAGU U AAUAAAA
1194	UUGAGUU A AUAAAAA

Table 36: RSV (N) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
9	AUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
21	UUGCUAA CUGAUGAGGCCGAAAGGCCGAA AGCCAUC
23	CUUUGCU CUGAUGAGGCCGAAAGGCCGAA AGAGCCA
24	ACUUUGC CUGAUGAGGCCGAAAGGCCGAA AAGAGCC
32	UCAACUU CUGAUGAGGCCGAAAGGCCGAA ACUUUGC
37	AUCAUUC CUGAUGAGGCCGAAAGGCCGAA ACUUGAC
45	UUGAGUG CUGAUGAGGCCGAAAGGCCGAA AUCAUUC
50	CUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGUGUAU
60	AGAAGUU CUGAUGAGGCCGAAAGGCCGAA AUCUUUG
65	AUGACAG CUGAUGAGGCCGAAAGGCCGAA AGUUGAU
66	GAUGACA CUGAUGAGGCCGAAAGGCCGAA AAGUUGA
70	GCUGGAU CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
73	UUUGCUG CUGAUGAGGCCGAAAGGCCGAA AUGACAG
82	GAUGGUG CUGAUGAGGCCGAAAGGCCGAA AUUUGCU
89	UCCGUUG CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
108	UCAAUAC CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
111	GUAUCAA CUGAUGAGGCCGAAAGGCCGAA ACUAUCU
113	GAGUAUC CUGAUGAGGCCGAAAGGCCGAA AUACUAU
117	UUAGGAG CUGAUGAGGCCGAAAGGCCGAA AUCAAUA
120	UAAUUAG CUGAUGAGGCCGAAAGGCCGAA AGUAUCA
123	UCAUAAU CUGAUGAGGCCGAAAGGCCGAA AGGAGUA
126	ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUUAGGA
127	CACAUCA CUGAUGAGGCCGAAAGGCCGAA AAUUAGG
146	ACUUAUU CUGAUGAGGCCGAAAGGCCGAA AUGUGUU
150	CAUAACU CUGAUGAGGCCGAAAGGCCGAA AUUGAUG
154	GCCACAU CUGAUGAGGCCGAAAGGCCGAA ACUUAUU
155	UGCCACA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
166	GAUUAAU CUGAUGAGGCCGAAAGGCCGAA ACAUGCC
167	UGAUUAA CUGAUGAGGCCGAAAGGCCGAA AACAUGC
169	UGUGAUU CUGAUGAGGCCGAAAGGCCGAA AUAACAU
170	CUGUGAU CUGAUGAGGCCGAAAGGCCGAA AAUAACA
173	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
186	UUAUGAU CUGAUGAGGCCGAAAGGCCGAA AGCAUCU
189	AAUUUAU CUGAUGAGGCCGAAAGGCCGAA AUUAGCA
192	GUGAAUU CUGAUGAGGCCGAAAGGCCGAA AUGAUUA
196	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
197	ACCCAGU CUGAUGAGGCCGAAAGGCCGAA AAUUUAU
205	ACCUAUU CUGAUGAGGCCGAAAGGCCGAA ACCCAGU
206	UACCUAU CUGAUGAGGCCGAAAGGCCGAA AACCCAG
209	ACAUACC CUGAUGAGGCCGAAAGGCCGAA AUUAACC
213	UAUAACA CUGAUGAGGCCGAAAGGCCGAA ACCUAUU

217	CGCAUAU	CUGAUGAGGCCGAAAGGCCGAA	ACAUACC
218	UCGCAUA	CUGAUGAGGCCGAAAGGCCGAA	AACAUAC
220	CAUCGCA	CUGAUGAGGCCGAAAGGCCGAA	AUAACAU
229	UAACCUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCGC
231	CCUAACC	CUGAUGAGGCCGAAAGGCCGAA	AGACAUC
235	UCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCUAGA
236	CUCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AACCUAG
254	GUAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
260	CUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUA
263	CAUCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGUAUUU
277	UACAUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCCGC
279	UUUACAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUCCC
284	UUGCUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUGAU
299	UUACAUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAU
305	GUGUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCUA
315	UCUUGAC	CUGAUGAGGCCGAAAGGCCGAA	AUGUGUU
318	AUGUCUU	CUGAUGAGGCCGAAAGGCCGAA	ACGAUGU
326	UUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
327	UUUCCAU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUCU
346	CACUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAU
347	ACACUUC	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
355	CAAUGUU	CUGAUGAGGCCGAAAGGCCGAA	ACACUUC
356	CCAAUGU	CUGAUGAGGCCGAAAGGCCGAA	AACACUU
361	GCUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AUGUUAA
370	AGUUGUU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGC
371	CAGUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUG
383	UGAUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAG
384	UUGAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
389	CAAUGUU	CUGAUGAGGCCGAAAGGCCGAA	AUUUGAA
395	CUAUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGUUGA
401	UAGAUUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCAA
406	UUUUCUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUAU
408	GAUUUUC	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCU
415	UUUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUCU
418	UUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAUUU
431	UUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
449	CUGGAGC	CUGAUGAGGCCGAAAGGCCGAA	ACCUCUC
453	UAUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUACC
460	AUGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGG
472	AUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCAUG
474	CAAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUCA
480	AUCCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGA
491	AUAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AUCAUCC
494	UACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUCA
496	UAUACAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAU
497	CUAUACA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUA
501	GCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAU
503	AUGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AUACAUA
511	UAUUACU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUGC

512	UUAUUAC	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUG
515	UAGUUAU	CUGAUGAGGCCGAAAGGCCGAA	ACUAAUG
518	AUUUAGU	CUGAUGAGGCCGAAAGGCCGAA	AUUACUA
522	GCUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUU
526	UGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	AUUUAGU
527	CUGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AAUUUAG
544	AAGACCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGUC
549	GCUGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGAU
551	CGGCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
552	ACGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
563	CUCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCACGG
564	GCUCUCC	CUGAUGAGGCCGAAAGGCCGAA	AAUCACG
573	ACAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGCUCUC
576	AGGACAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAGCU
581	UUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAU
584	CAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGGACAU
603	CCUUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACGUUUC
604	GCCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AACGUUU
613	GGGUAGU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUU
614	UGGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCUU
617	CCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGC
629	UGUUGGC	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCU
640	UUCAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUU
641	CUUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
643	CACUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
652	UUUUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUC
653	GUUUUUC	CUGAUGAGGCCGAAAGGCCGAA	AACACUU
663	AAGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUU
670	AUCUAUA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGG
671	CAUCUAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGG
672	ACAUCUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGG
674	AAACAUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGU
680	GAACAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCUA
681	UGAACAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUCU
682	AUGAACA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUC
683	AAUGAAC	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
686	CAAAAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAAA
687	CCAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AACAAAA
690	AUACCAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAACA
691	UAUACCA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAC
692	CUAUACC	CUGAUGAGGCCGAAAGGCCGAA	AAAUGAA
696	UGUGCUA	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAA
698	AUUGUGC	CUGAUGAGGCCGAAAGGCCGAA	AUACCAA
706	GGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUGC
708	CUGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
709	UCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUG
711	CCUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAU
726	UCAACUC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCCA
731	UCCCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAC

740	CUGCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCCCUU
741	CCUGCAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCCCU
742	UCCUGCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCC
743	AUCCUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCC
751	CAUAAAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGC
754	AUUCAUA	CUGAUGAGGCCGAAAGGCCGAA	ACAAUCC
755	CAUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AACAAUC
756	GCAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAACAAU
766	UGCACCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUU
787	CCACCGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAC
788	CCCACCG	CUGAUGAGGCCGAAAGGCCGAA	AACAUCA
800	UUGCUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCCC
802	UUUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGACUCC
803	AUUUUGC	CUGAUGAGGCCGAAAGGCCGAA	AAGACUC
811	UUUAACU	CUGAUGAGGCCGAAAGGCCGAA	AUUUUGC
815	UAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAUU
816	AUAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACUGAU
822	AACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUA
824	CUAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUU
825	CCUAACA	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
829	AUGUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAU
830	CAUGUCC	CUGAUGAGGCCGAAAGGCCGAA	AACAUAA
840	UGCACAC	CUGAUGAGGCCGAAAGGCCGAA	AGCAUGU
866	CCUCAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUGUU
869	AAACCUC	CUGAUGAGGCCGAAAGGCCGAA	ACAACUU
875	AUUCAUA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAA
876	UAUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AACCUCA
877	AUAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAACCUC
883	UUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCAUA
895	ACCACCC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUG
913	AUGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGC
914	UAUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAUCCUG
916	UAUAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUCC
921	UUCAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
923	UGUUCAA	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGU
925	GUUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AUAUAUG
943	UAAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
946	AGAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AUGAUGC
947	AAGAUAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAUG
949	CAAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUGA
950	UCAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUAAUG
952	AGUCAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUAA
954	UGAGUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAUAAU
955	UUGAGUC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUAA
960	GGAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGUCAAA
964	GUGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGU
965	AGUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGAG
966	AAGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
969	GAGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAAU

973	ACUGGAG CUGAUGAGGCCGAAAGGCCGAA AGUGAGG
974	CACUGGA CUGAUGAGGCCGAAAGGCCGAA AAGUGAG
976	UACACUG CUGAUGAGGCCGAAAGGCCGAA AGAAGUG
983	CUAAUAC CUGAUGAGGCCGAAAGGCCGAA ACACUGG
986	UGCCUAA CUGAUGAGGCCGAAAGGCCGAA ACUACAC
988	AUUGCCU CUGAUGAGGCCGAAAGGCCGAA AUACUAC
989	CAUUGCC CUGAUGAGGCCGAAAGGCCGAA AAUACUA
1007	UUAUGCC CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
1013	CUCCCAU CUGAUGAGGCCGAAAGGCCGAA AUGCCUA
1024	ACCUCUG CUGAUGAGGCCGAAAGGCCGAA ACUCCCC
1032	CUCGGUG CUGAUGAGGCCGAAAGGCCGAA ACCUCUG
1044	AGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUUCCUC
1050	UCAUAUA CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
1052	CAUCAUA CUGAUGAGGCCGAAAGGCCGAA AGAUCUU
1054	UGCAUCA CUGAUGAGGCCGAAAGGCCGAA AUAGAUC
1072	UUCAGCA CUGAUGAGGCCGAAAGGCCGAA AUGCCUU
1085	UUUCUUU CUGAUGAGGCCGAAAGGCCGAA AGUUGUU
1103	UGUAGUU CUGAUGAGGCCGAAAGGCCGAA AUCACAC
1104	CUGUAGU CUGAUGAGGCCGAAAGGCCGAA AAUCACA
1108	UACACUG CUGAUGAGGCCGAAAGGCCGAA AGUUAAU
1115	AGUCUAG CUGAUGAGGCCGAAAGGCCGAA ACACUGU
1118	UCAAGUC CUGAUGAGGCCGAAAGGCCGAA AGUACAC
1123	UGCUGUC CUGAUGAGGCCGAAAGGCCGAA AGUCUAG
1139	UAGCCUC CUGAUGAGGCCGAAAGGCCGAA AGUUCUU
1146	UGUUUGA CUGAUGAGGCCGAAAGGCCGAA AGCCUCU
1148	GAUGUUU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
1155	UUAAGCU CUGAUGAGGCCGAAAGGCCGAA AUGUUUG
1160	UUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1161	UUUGGAU CUGAUGAGGCCGAAAGGCCGAA AAGCUGA
1164	UCUUUUG CUGAUGAGGCCGAAAGGCCGAA AUUAAGC
1173	ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUCUUUU
1181	AAAGCUC CUGAUGAGGCCGAAAGGCCGAA ACAUCAU
1187	UAACUCA CUGAUGAGGCCGAAAGGCCGAA AGCUCUA
1188	UUAACUC CUGAUGAGGCCGAAAGGCCGAA AAGCUCU
1193	UUUUAUU CUGAUGAGGCCGAAAGGCCGAA ACUCAAA
1194	UUUUUAU CUGAUGAGGCCGAAAGGCCGAA AACUCAA

Table 37: RSV (1B) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence	Substrate
70	CUGUGAUC AGAA GUCUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAGACU GAU GAUCACAG
91	CAAGUGAC AGAA GUCUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGAGACC GUU GUCACUUG
472	CAGGCUCC AGAA GGACUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAGUCCA GAU GGAGCCUG

Table 38: RSV (N) HP Ribozyme/Substrate Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
476	AUCCCACA AGAA GGAGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCUCCU GAU UGUGGGAU
540	AAGACCAG AGAA GUCCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGGGACA GAU CUGGUCUU
554	CUAAUCAC AGAA GUAAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCUUACA GCC GUGAUUAG
636	UUCAUAGA AGAA GUUGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCAACA GCU UCUAUGAA
998	CCUAGGCC AGAA GCAUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAAUGCU GCU GGCCUAGG
1156	UUGGAUUA AGAA GAUGUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AACAUCA GCU UAAUCCAA

Table 39: Large-Scale Synthesis

Sequence	Activator [Added/Final] (min)	Amidite [Added/Final] (min)	Time*	% Full Length Product
A_9T	T [0.50/0.33]	[0.1/0.02]	15 m	85
A_9T	S [0.25/0.17]	[0.1/0.02]	15 m	89
$(GGU)_3GGT$	T [0.50/0.33]	[0.1/0.02]	15 m	78
$(GGU)_3GGT$	S [0.25/0.17]	[0.1/0.02]	15 m	81
C_9T	T [0.50/0.33]	[0.1/0.02]	15 m	90
C_9T	S [0.25/0.17]	[0.1/0.02]	15 m	97
U_9T	T [0.50/0.33]	[0.1/0.02]	15 m	80
U_9T	S [0.25/0.17]	[0.1/0.02]	15 m	85
A (36-mer)	T [0.50/0.33]	[0.1/0.02]	15/15m	21
A (36-mer)	S [0.25/0.17]	[0.1/0.02]	15/15 m	25
A (36-mer)	S [0.50/0.24]	[0.1/0.03]	15/15 m	25
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	15/15 m	38
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	10/5 m	42

*Where two coupling times are indicated the first refers to RNA coupling and the second to 2'-*O*-methyl coupling. S = 5-*S*-Ethyltetrazole, T = tetrazole activator. A is 5' -ucu ccA UCU GAU GAG GCC GAA AGG CCG AAA Auc ccu -3' where lowerecase represents 2'-*O*-methylnucleotides.

Table 40: Base Deprot ction

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
iBu(GGU)$_4$	NH_4OH/EtOH	16 h	55	62.5
	MA	10 m	65	62.7
	AMA	10 m	65	74.8
	MA	10 m	55	75.0
	AMA	10 m	55	77.2
iPrP(GGU)$_4$	NH_4OH/EtOH	4 h	65	44.8
	MA	10 m	65	65.9
	AMA	10 m	65	59.8
	MA	10 m	55	61.3
	AMA	10 m	55	60.1
C_9U	NH_4OH/EtOH	4 h	65	75.2
	MA	10 m	65	79.1
	AMA	10 m	65	77.1
	MA	10 m	55	79.8
	AMA	10 m	55	75.5
A (36-mer)	NH_4OH/EtOH	4 h	65	22.7
	MA	10 m	65	28.9

Table 41: 2'-*O*-Alkylsilyl Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
A_9T	TBAF	24 h	20	84.5
	1.4 M HF	0.5 h	65	81.0
$(GGU)_4$	TBAF	24 h	20	60.9
	1.4 M HF	0.5 h	65	67.8
C_{10}	TBAF	24 h	20	86.2
	1.4 M HF	0.5 h	65	86.1
U_{10}	TBAF	24 h	20	84.8
	1.4 M HF	0.5 h	65	84.5
B (36-mer)	TBAF	24 h	20	25.2
	1.4 M HF	1.5 h	65	30.6
A (36-mer)	TBAF	24 h	20	29.7
	1.4 M HF	1.5 h	65	30.4

B is 5'- UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU -3'.

Table 42 : *NMR Data for UC Dimers containing Phosphorothioate Linkage*

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3524	ribo	2 x 3 s	10.4	2 x 100 s	95.9
3525	ribo	2 x 3 s	10.4	2 x 75 s	92.6
3530	ribo	2 x 3 s	10.4	2 x 75 s	92.1
3526	ribo	1 x 5 s	08.6	1 x 300 s	100.0
3578	ribo	1 x 5 s	08.6	1 x 250 s	100.0
3529	ribo	1 x 5 s	08.6	1 x 150 s	73.7

Table 43: *NMR Data for 15-mer RNA containing Phosphorothioate Linkages*

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3581	ribo	1 x 5 s	08.6	1 x 250 s	99.6
3663	ribo	2 x 4 s	13.8	2 x 300 s	100.0
3582	2'-*O*-Me	1 x 5 s	08.6	1 x 250 s	99.7
3668	2'-*O*-Me	2 x 4 s	13.8	2 x 300 s	99.8
3682	2'-O-Me	1 x 5 s	08.6	1 x 300 s	99.8

Table 44. Kinetics of Self-Processing *In Vitro*

Self-Processing Constructs	k (min^{-1})*
HH	1.16 ± 0.08
HDV	0.56 ± 0.15
HP(GC)	0.36 ± 0.06
HP(GU)	0.054 ± 0.003

* k represents the unimolecular rate constant for ribozyme self-cleavage determined from a non-linear, least-squares fit (KaleidaGraph, Synergy Software, Reeding, PA) to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt}(1\text{-}e^{-kt})$$

The equation describes the extent of ribozyme processing in the presense of ongoing transcription (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977) as a function of time (t) and the unimolecular rate constant for cleavage (k). Each value of k represents the average (± range) of values determined from two experiments.

Table 45

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A \times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-*O*-Me-U	4	260	650
3	U4 = 2'=CH_2-U	6.5	120	180
4	U7 = 2'=CH_2-U	8	280	350
5	U4 & U7 = 2'=CH_2-U	9.5	120	130
6	U4 = 2'=CF_2-U	5	320	640
7	U7 = 2'=CF_2-U	4	220	550
8	U4 & U7 = 2'=CF_2-U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH_2-U	10	500	500
19	U7 = 2'-NH_2-U	5	500	1000
20	U4 & U7 = 2'-NH_2-U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

CLAIMS

What is claimed is:

1. An enzymatic nucleic acid molecule which cleaves ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF-α mRNA sites shown in Table 23, 25, 27, or 28, CML associated mRNA selected from those identified as SEQ. ID NOS 1-25, or RSV mRNA or RSV genomic RNA in a region selected from the group consisting of 1C, 1B and N.

2. The enzymatic nucleic acid molecule of claim 1, the binding arms of which contain sequences complementary to any one of the sequences defined in any of those in Tables 2, 3, 6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36, and 37..

3. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hammerhead motif.

4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said RNA molecule is in a hairpin, hepatitis delta virus, group 1 intron, *Neurospora VS* RNA or RNaseP RNA motif.

5. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 12 and 100 bases complementary to said mRNA or genomic RNA.

6. The enzymatic nucleic acid molecule of claim 5 comprising between 14 and 24 bases complementary to said mRNA or genomic RNA.

7. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 5 and 23 bases complementary to said mRNA or genomic RNA.

8. The enzymatic nucleic acid molecule of claim 7 comprising between 10 and 18 bases complementary to said mRNA or genomic RNA.

9. An enzymatic nucleic acid molecule consisting essentially of a sequence selected from the group of those shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38.

10. A mammalian cell including an enzymatic nucleic acid molecule of claims 1 or 2.

11. The cell of claim 10, wherein said cell is a human cell.

12. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.

13. A mammalian cell including an expression vector of claim 12.

14. The cell of claim 13, wherein said cell is a human cell.

15. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF-α, or RSV by administering to a patient an enzymatic nucleic acid molecule of claim 1 or 2.

16. A method for treatment of a pathological condition related to th mRNA level of ICAM-1, IL-5, *rel A*, TNF-α, or RSV by administering to a patient an expression vector of claim 12.

17. The method of claims 15 or 16, wherein said patient is a human.

18. The method of claim 17 wherein said condition is selected from the group consisting of atherosclerosis, myocardial infraction, stroke, restenosis, heart diseases, cancer, rheumatoid arthritis, asthma, reperfusion injury, inflammatory or autoimmune disorders, transplant rejection, myocardial ischemia, stroke, psoriasis, Kawasaki disease, HIV and AIDS, and septic shock.

19. A nucleoside selected from the group consisting of 5'-C-alkylnucleoside, 2'-deoxy-2'-alkylnucleoside, nucleoside 5'-deoxy-5'-dihalo-methylphosphonate, nucleoside 5'-deoxy-5'-difluoro-methylphosphonate, nucleoside 3'-deoxy-3'-dihalo-methylphosphonate, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

20. A nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

21. A nucleotide triphosphate comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

22. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in a talo configuration.

23. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in an allo configuration.

24. An oligonucleotide comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

25. An oligonucleotide comprising a moiety having the formula:

wherein B is a nucleotide base or hydrogen; R1, R2 and R3 independently is selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.

26. An oligonucleotide comprising a 3'-amido or peptido group.

27. An oligonucleotide comprising a 5'-amido or peptido group.

28. The oligonucleotide of claim 24, 25, 26, or 27 having enzymatic activity.

29. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having an alkyl group at its 5'-position or 2'-position.

30. Method for conversion of a protected allo sugar to a protected talo sugar, comprising the step of contacting said protected allo sugar with triphenyl phosphine, diethylazodicarbcxylate, p-nitrobenzoic acid under inversion causing conditions to provide said protected talo sugar.

31. Method for the synthesis of a nucleoside 5' or a 3'-dihalo-methylphosphonate comprising the step of condensing a difluoromethylphosphonate-containing sugar with a pyrimidine or purine under conditions suitable for forming a nucleoside 5'- or 3'-difluoromethylphosphonate.

32. The oligonucleotide of claim 3, wherein the normal hammerhead U4 and/or U7 positions are substituted with 2'-NH-amino acid.

33. A method for the synthesis of RNA comprising the step of providing 5-*S*-alkyltetrazole at a delivered 0.1-1.0 M concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.

34. A method for the synthesis of RNA comprising the step of providing 5-*S*-alkyltetrazole at 0.15-0.35 M effective, or final, concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.

35. A method for the deprotection of RNA comprising the step of providing alkylamine (MA) or NH_4OH/alkylamine (AMA) at between 60°C - 70°C for 5 to 15 minutes to remove any exocyclic amino protecting groups from protected RNA; wherein said alkyl is selected from the group consisting of methyl, ethyl, propyl and butyl.

36. A method for the deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine•hydrogen fluoride (aHF•TEA) trimethylamine or disopropylethylamine at between 60 °C-70 °C for 0.25-24 h.

37. A method for the purification of an RNA molecule by passing said enzymatic RNA molecule over an HPLC column, wherein said HPCC column is an anion exchange chromatography column.

38. Method for one pot deprotection of RNA comprising, contacting a protected base with anhydrous methyl amine at between 60 °C-70 °C for at least 5 min, cooling the resulting mixture and contacting said mixture with TEA-3HF reagents under conditions which remove a protecting group of the 2'-hydroxyl position.

39. Method for synthesizing RNA containing a phosphorothioate linkage comprising the step of contacting 6-10 equivalents of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) with the growing RNA chain for 5 seconds with a reaction time of at least 300 seconds.

40. Method of synthesizing RNA containing a phosphorothioate linkage comprising the step of achieving coupling with 5-*S*-ethyltetrazole or 5-*S*-methyltetrazole prior to sulfurization.

41. Method of claims 38, 39 or 40 wherein said RNA is enzymatically active.

42. Method for synthesizing 2'-deoxy-2'-amino-nucleoside phosphoramidite, comprising the step of protecting the 2'-amino group with a N-phtaloyl group.

43. The method of claim 42 wherein the said nucleoside lacks a base.

44. Method for synthesis of RNA comprising the step of: protecting the 2'-position of a nucleotide during said synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group.

45. Method for covalently linking a SEM group to the 2'-position of a nucleotide, comprising the step of: contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions.

46. The method of claim 45, wherein said conditions comprise dibutyltin oxide and tetrabutylammonium fluoride and SEM-Cl.

47. Method for removal of an SEM group from a nucleoside molecule or an oligonucleotide, comprising the step of: contacting said molecule or oligonucleotide with boron trifluoride etherate ($BF_3 \bullet OEt_2$) under SEM removing conditions.

48. The method of claim 57 wherein said (BF_3•OEt_2) is provided in acetonitrile.

49. One or more vectors comprising

a first nucleic acid sequence encoding a first ribozyme having intramolecular or intermolecular cleaving activity, said first ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif;

and a second nucleic acid sequence encoding a second ribozyme having intermolecular cleaving activity, said Second ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif and said second nucleic acid being flanked by other nucleic acid sequences encoding RNA which is cleaved by said first ribozyme to release said second ribozyme from RNA encoded by said vector;

wherein said first and second nucleic acid sequences may be on the same or separate nucleic acid molecules, and said vector encodes mRNA or comprises RNA which lacks secondary structure which reduces release of said second ribozyme by more than 20%.

50. Cell comprising the vector of claim 49.

51. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs.

52. The RNA molecule of claim 51, wherein said molecule is transcribed by a RNA polymerase III based promoter system.

53. The RNA molecule of claim 51, wherein said molecule is transcribed by a type 2 pol III promoter system.

54. The RNA molecule of claim 51, wherein said molecule is a chimeric tRNA.

55. The RNA molecul of claim 53, said RNA having A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases.

56. The RNA molecule of claim 53, wherein said desired RNA molecule is at the 3' end of said B box.

57. The RNA molecule of claim 53, wherein said desired RNA molecule is in between the said A and the B box.

58. The RNA molecule of claim 53, wherein said desired RNA molecule includes said B box.

59. The RNA molecule of claim 51, wherein said desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA.

60. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 12 bases of said 3' region.

61. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 15 bases of said 3' region.

62. DNA vector encoding the RNA molecule of claim 51

63. The vector of claim 62, wherein said vector is derived from an AAV or adeno virus.

64. RNA vector encoding the RNA molecule of claim 51.

65. The vector of claim 64, wherein said vector is derived from an alpha virus or retro virus.

66. The vector of claim 62 wherein the portions of the vector encoding said RNA function as a RNA pol III promoter.

67. Cell comprising the vector of claim 62.

68. Cell comprising the vector of claim 53.

69. Cell comprising the RNA of claim 51.

70. M thod to provide a desired RNA molecule in a cell, comprising introducing said molecule into said cell a RNA comprising a desired RNA molecule, having a 5' terminus able to base pair with at least 8 bases of a 3' region of said RNA molecule.

71. The method of claim 70, wherein said introducing comprises providing a vector encoding said RNA molecule.

72. Hammerhead ribozyme having 2 or 3 base pairs in stem II with an interconnecting loop of 4 or more bases between said base pairs.

73. Hairpin ribozyme lacking a substrate moiety, comprising at least six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said ribozyme comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said ribozyme can cleave and/or ligate said separate RNA(s) in *trans*.

74. The ribozyme of claim 73, wherein said ribozyme comprises six bases in helix 2.

75. The ribozyme of claim 73, having the structure of Fig. 3, wherein each N and N' is independently any base and each dash may represent a hydrogen bond, r is 1-20, q is 2-20, o is 0 - 20, n is 1 - 4, and m is 1 - 20.

76. Method for increasing the activity of a hairpin ribozyme by providing one or more bases 3' of helix 3 able to base-pair with a substrate RNA to form a helix 5.

77. Trans-cleaving Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.

78. Trans-ligating Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.

79. The ribozyme of claim 73 having the structure of Fig. 73.

80. The ribozyme of claim 73 having the structure of Fig. 74.

81. A cell including the ribozyme of any of claims 73-80.

82. An expr ssion vector comprising nucl ic acid encoding the ribozyme of any of claims 73-80, in a manner which allows expression of that ribozym within a cell.

83. A cell including an expression vector of claim 82.

84. Method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule, comprising the steps of:

contacting said nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid able to form a duplex or triplex molecule with said nucleic acid molecule, wherein formation of said duplex or triplex molecule directly, or after nucleic acid repair *in vivo*, causes at least one base in said nucleic acid molecule to be chemically modified to functionally alter the nucleotide base sequence of said nucleic acid sequence.

85. The method of claim 84, wherein said oligonucleotide is of a length sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine in an RNA molecule.

86. The method of claim 84, wherein said oligonucleotide comprises an enzymatic nucleic acid molecule which is active to chemically modify a base.

87. The method claim 84, wherein said nucleic acid molecule is DNA or RNA.

88. The method of claim 84, wherein said oligonucleotide comprises a chemical mutagen.

89. The method of claim 88, wherein said mutagen is nitrous acid.

90. The method of claim 84 wherein said oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

91. The method of claim 84, wherein an endogenous mammalian editing system is co-opted to cause said chemical modification.

92. Method for introduction of enzymatic nucleic acid into a cell or tissue, comprising the steps of;

providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

and contacting said complex with said cell or tissue under conditions in which said enzymatic nucleic acid molecule is produced in said cell or tissue.

93. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

providing a complex of a first nucleic acid molecule encoding said desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

and contacting said complex with said cell or tissue under conditions in which said desired acid molecule is produced in said cell or tissue.

94 Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

providing a complex of a first nucl ic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired

structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

and wherein said second nucleic acid further comprises a localization factor;

and contacting said complex with said cell or tissue under conditions in which said desired nucleic acid molecule is produced in said cell or tissue.

95. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

96. Complex of a first nucleic acid molecule encoding a desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

97. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said

first nucleic acid under said conditions, and wherein said second nucleic acid further comprises a localization factor.

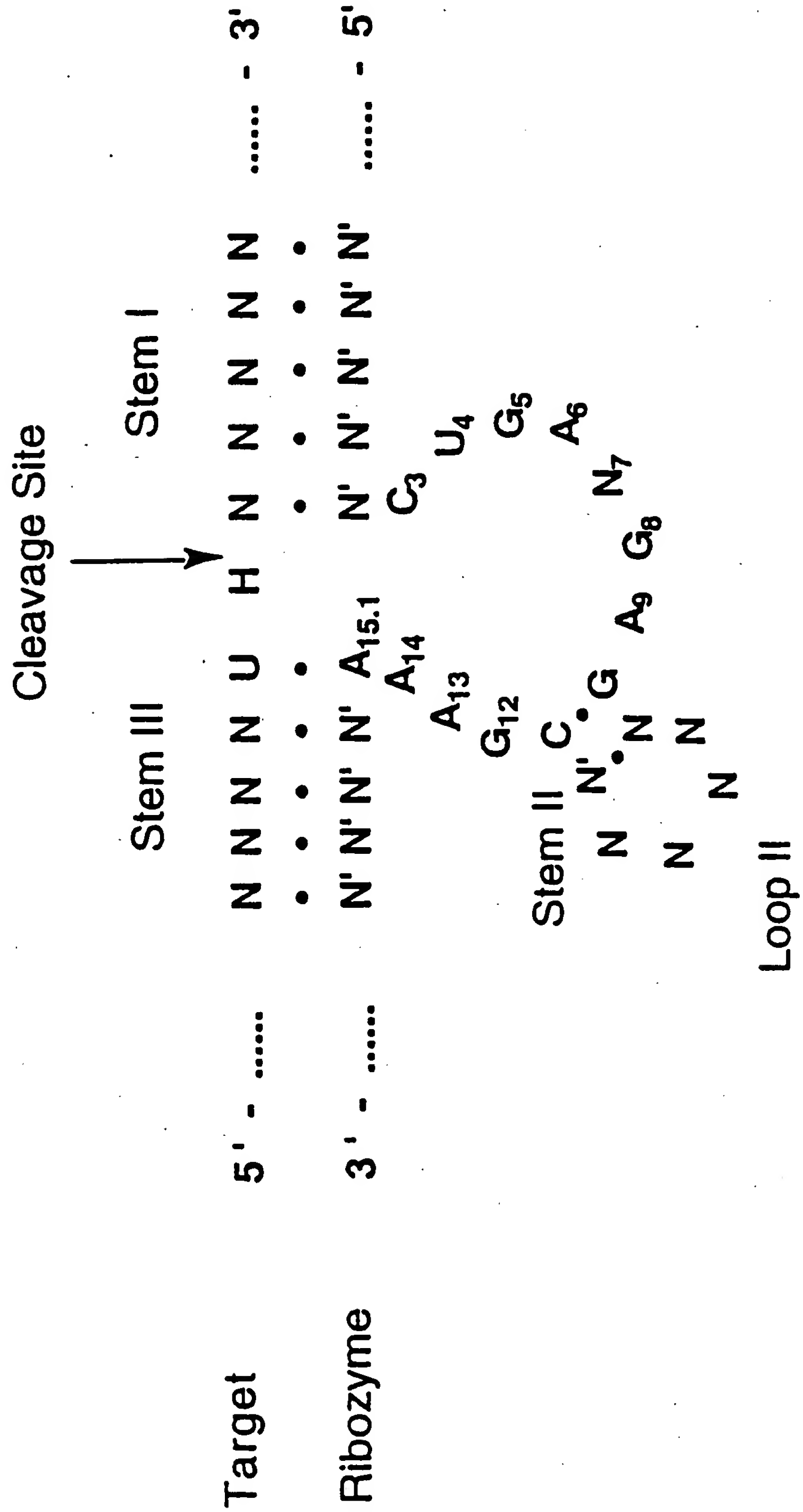

Cleavage Site
Stem I
Stem II
Stem III
Loop II
Target
Ribozyme
5' - ...... N N N N U H N N N N N ...... - 3'
3' - ...... N' N' N' N' A15.1 N' N' N' N' N' ...... - 5'
C3 U4 G5 A6 N7 G8 A9
A14 A13 G12
C G
N' N
N N
N N

FIG. 1.

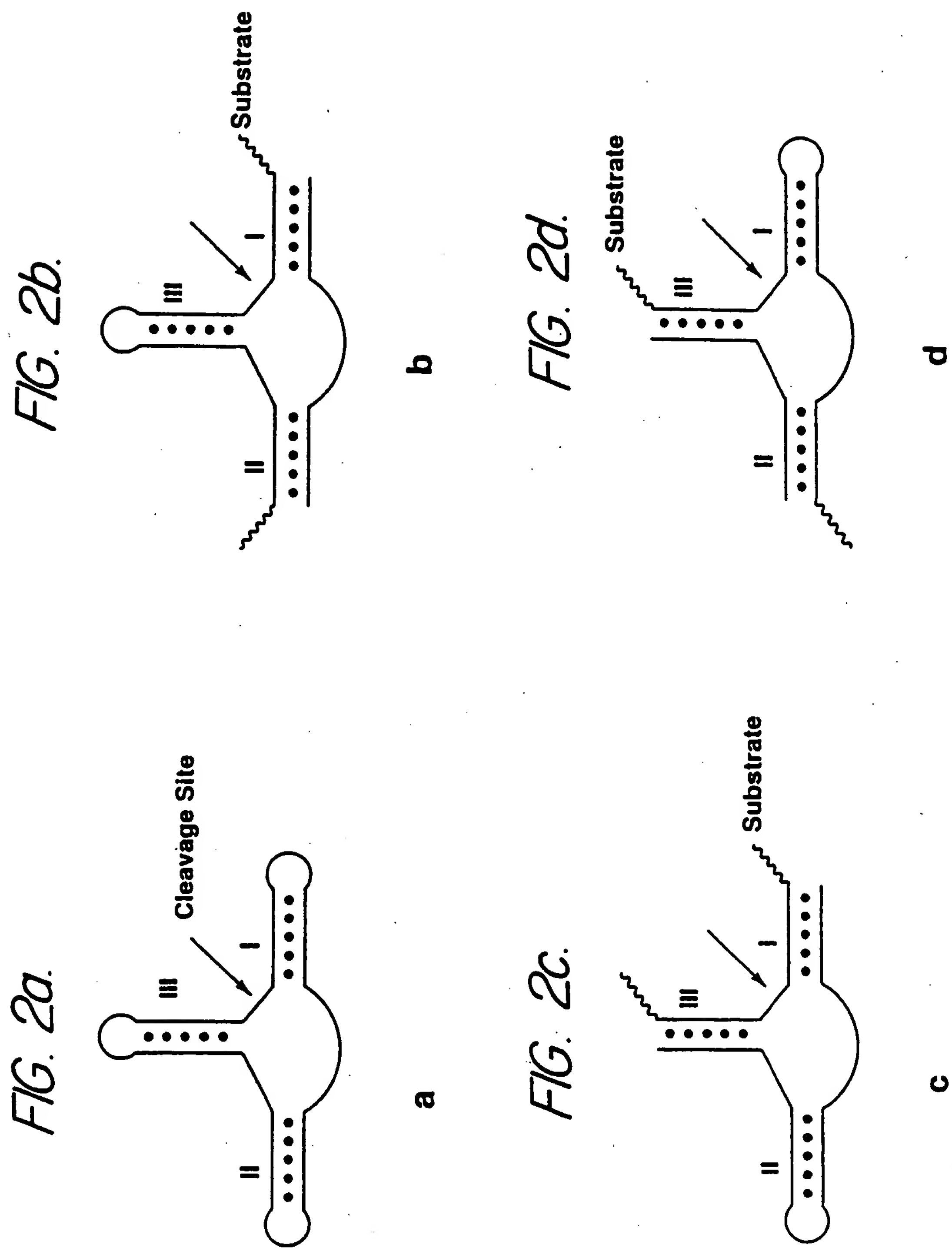


FIG. 2a.

FIG. 2b.

FIG. 2c.

FIG. 2d.

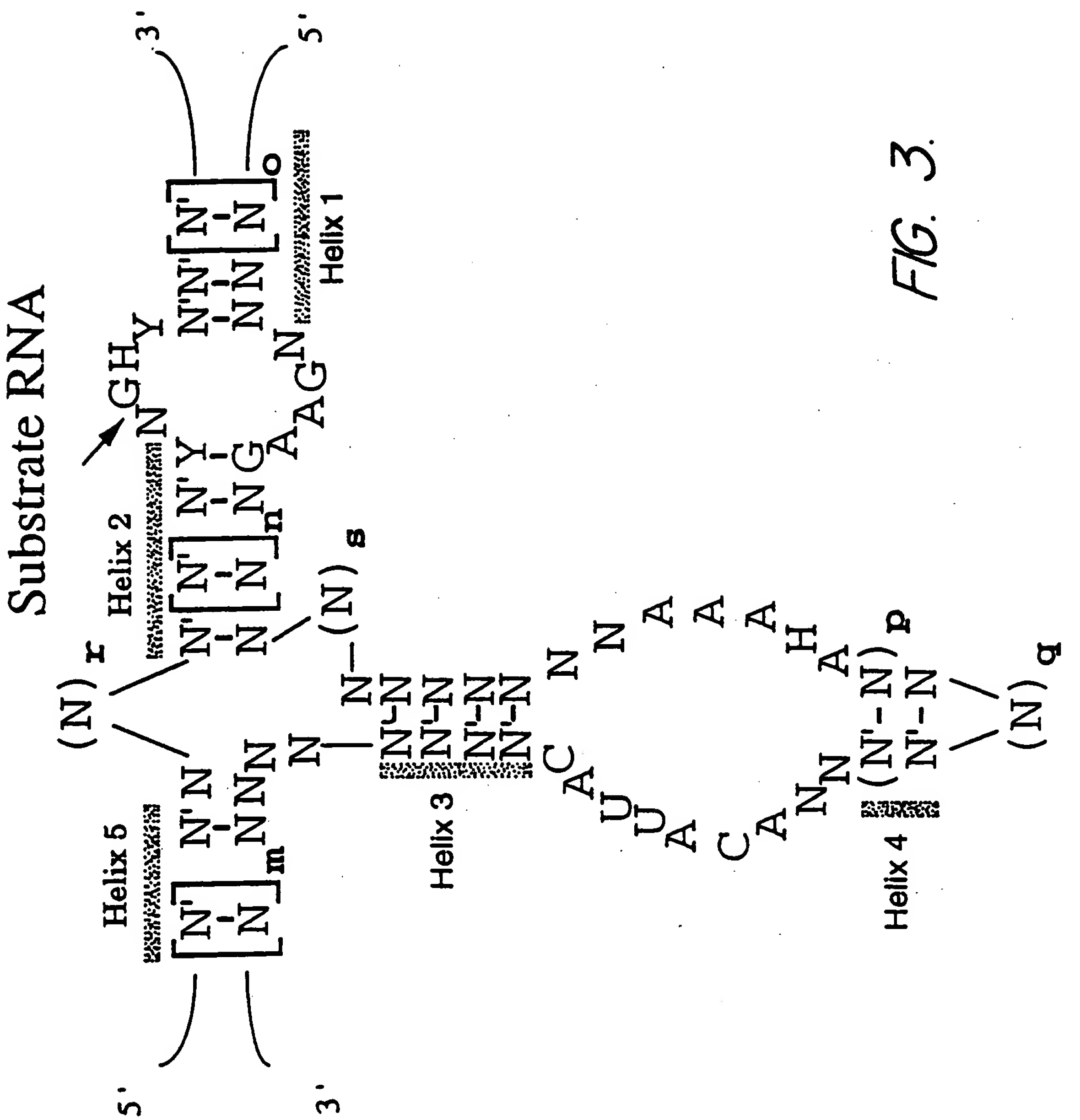


FIG. 3

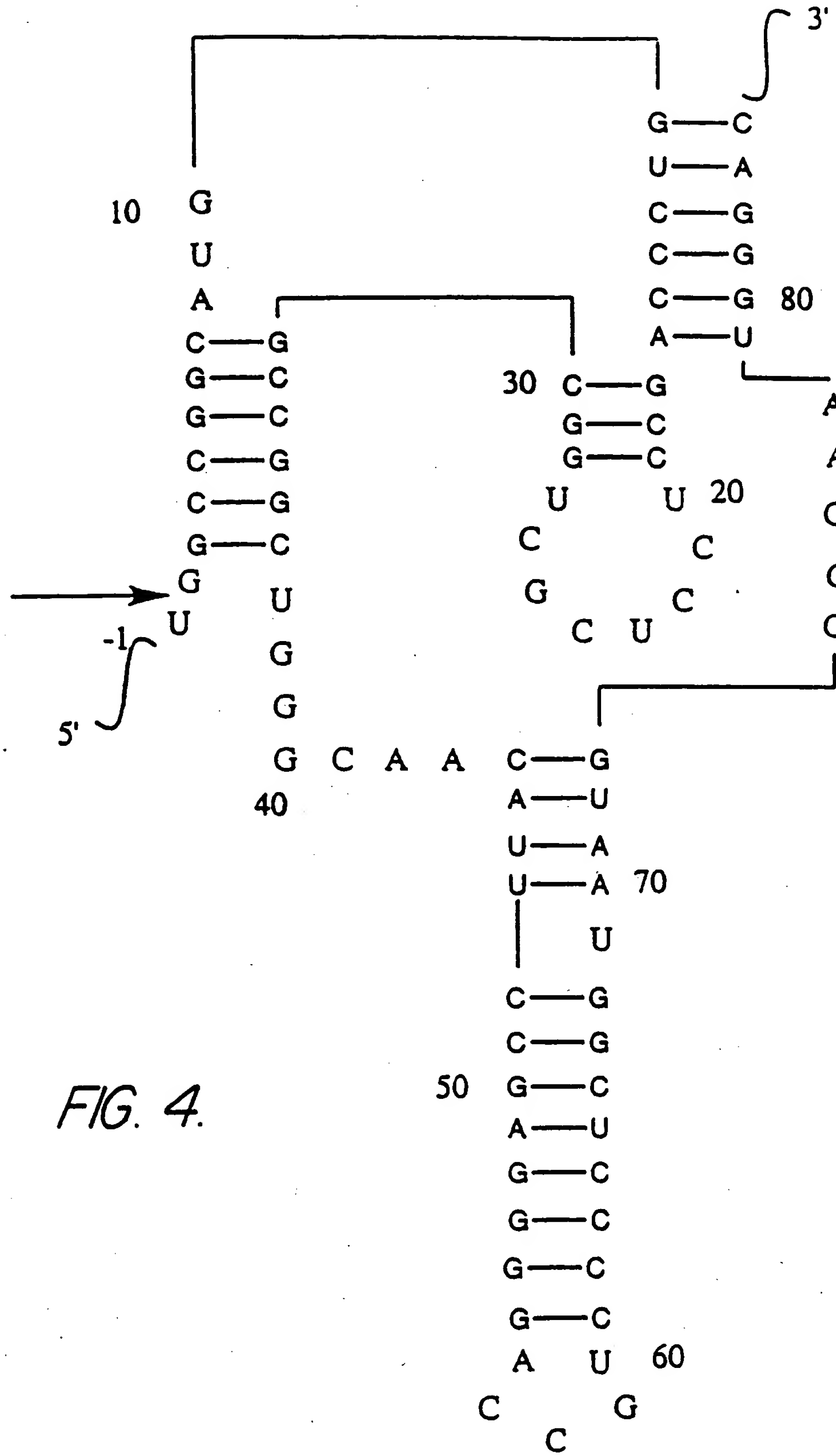


FIG. 4.

FIG. 5.

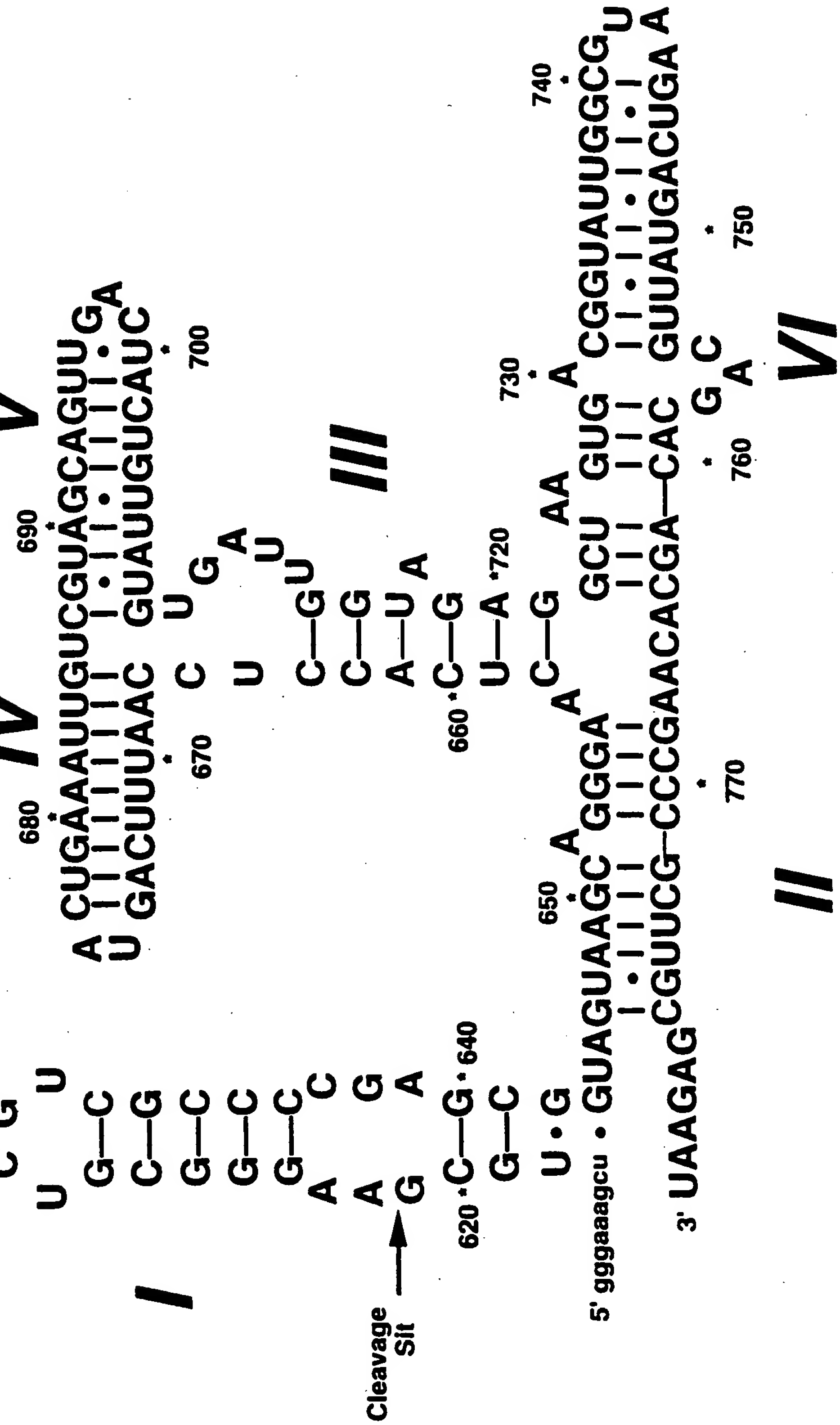
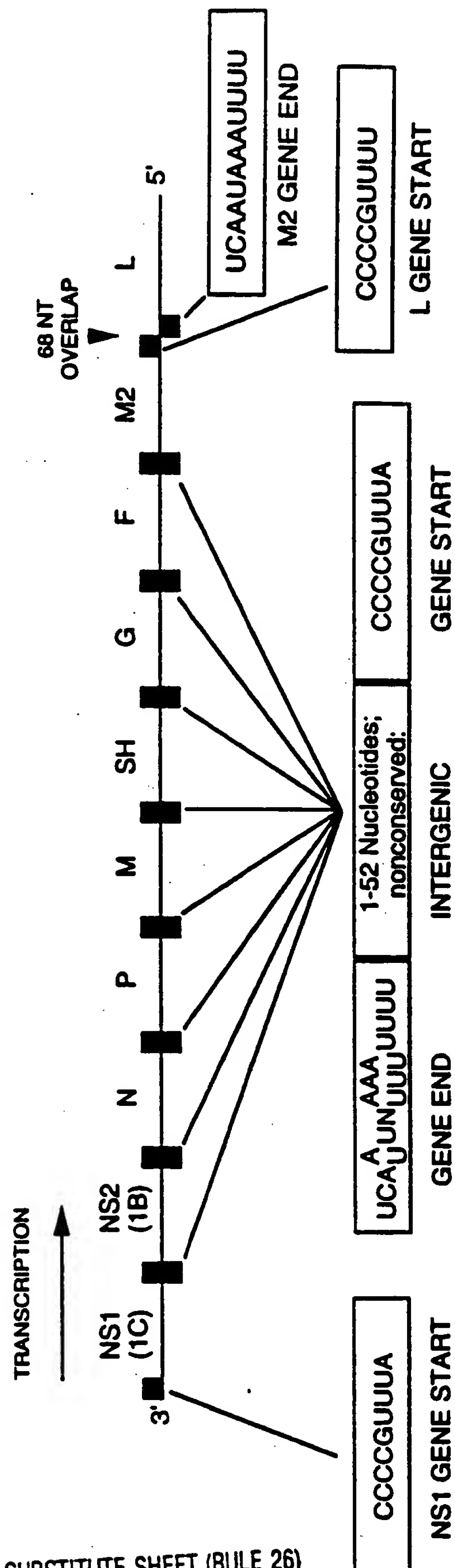
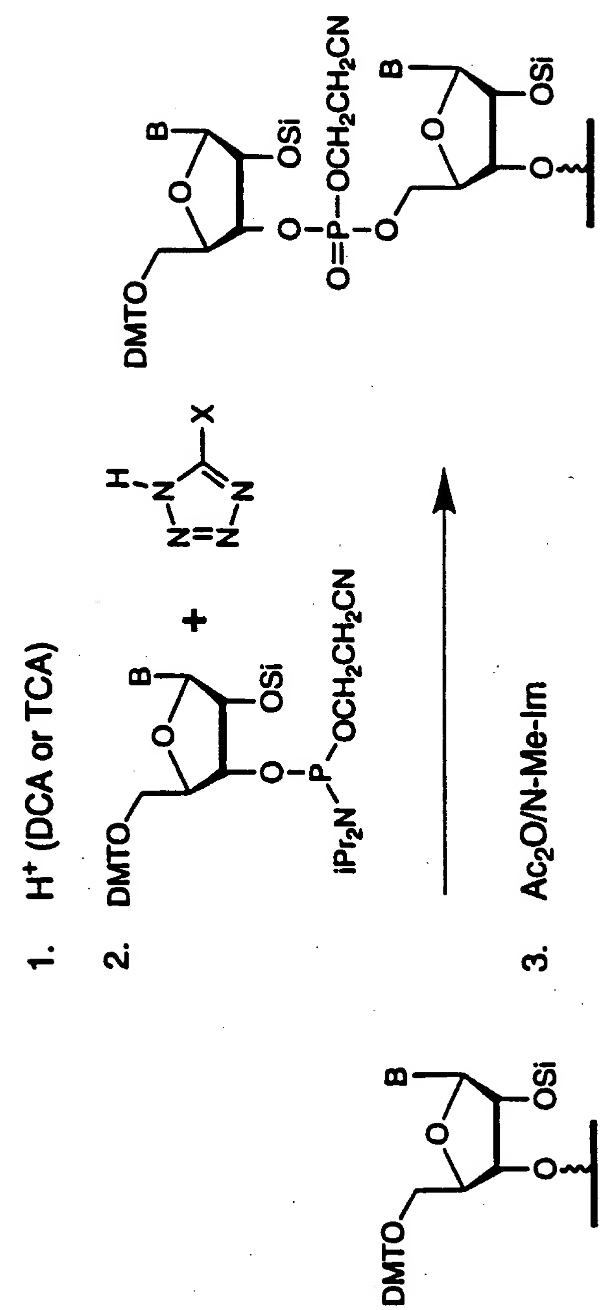

I
II
III
IV
V
VI
Cleavage Slt
5' gggaaagcu
3' UAAGAG
620
630
640
650
660
670
680
690
700
720
730
740
750
760
770

FIG. 6.

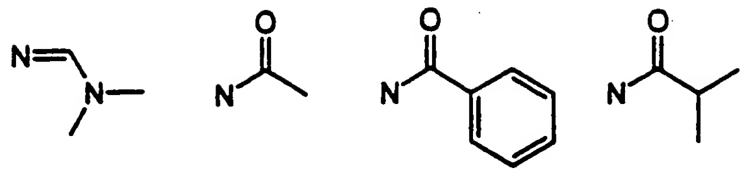


Adapted from Virology, Second Edition, Edited by B.N. Fields, 1990.

FIG. 7.



DMTO
B
O
OSi
O
1. H+ (DCA or TCA)
2. DMTO
B
O
OSi
O
P
iPr2N
OCH2CH2CN
+
N=N
N
N
H
X
3. Ac2O/N-Me-Im
4. I2/H2O
DMTO
B
O
OSi
O
O=P-OCH2CH2CN
O
B
O
OSi
O



DMF Ac Bz *i*-Bu

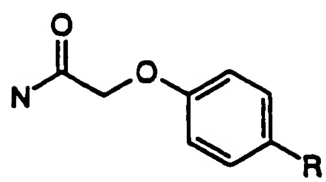


FIG. 8.

R = H = PAC

R = tBu = TAC

R = iPr = iPPAC

FIG. 9.

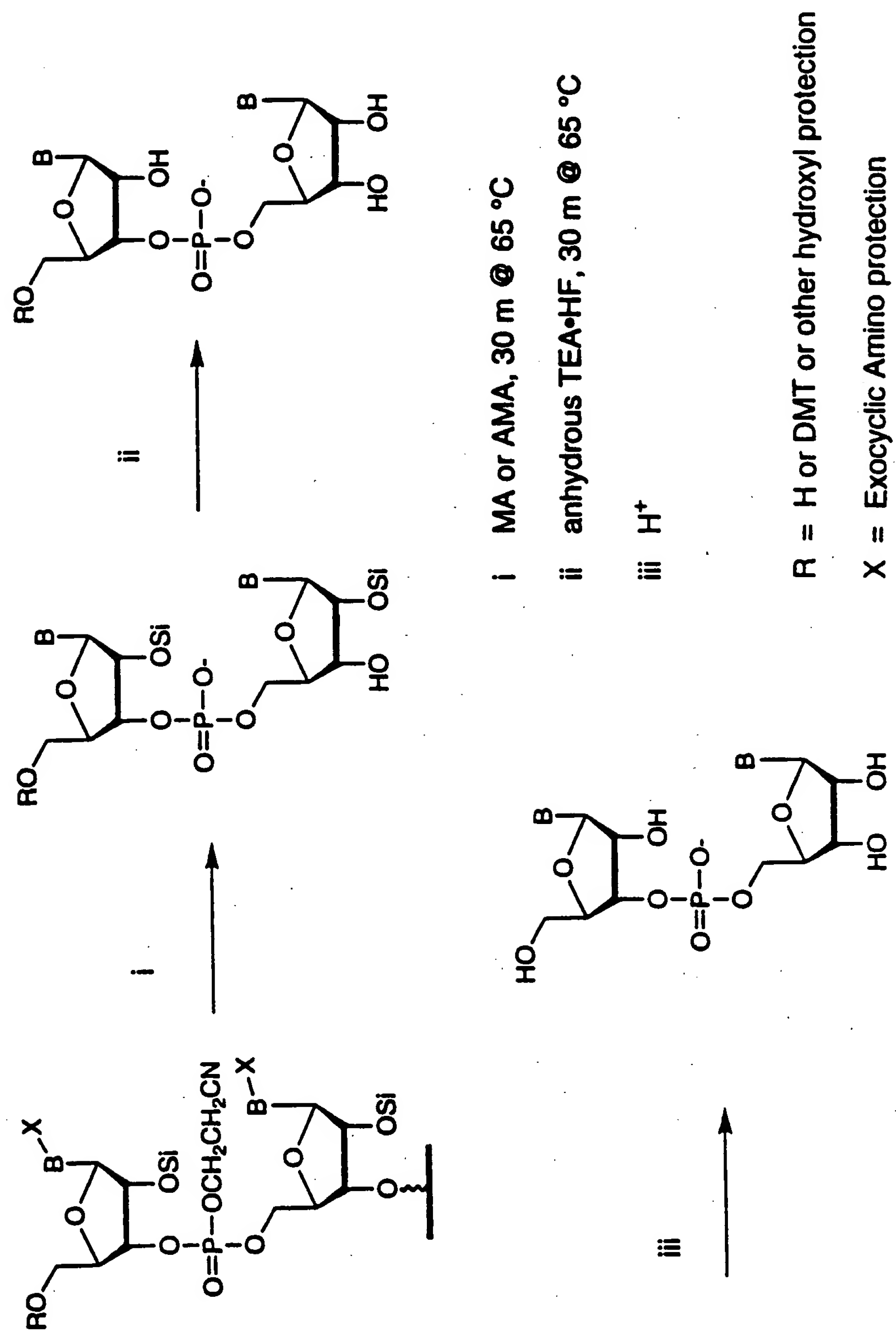

i
ii
iii
RO
HO
B
B–X
O
OSi
OH
O=P–OCH$_2$CH$_2$CN
O=P–O-
i MA or AMA, 30 m @ 65 °C
ii anhydrous TEA•HF, 30 m @ 65 °C
iii H$^+$
R = H or DMT or other hydroxyl protection
X = Exocyclic Amino protection

FIG. 10.

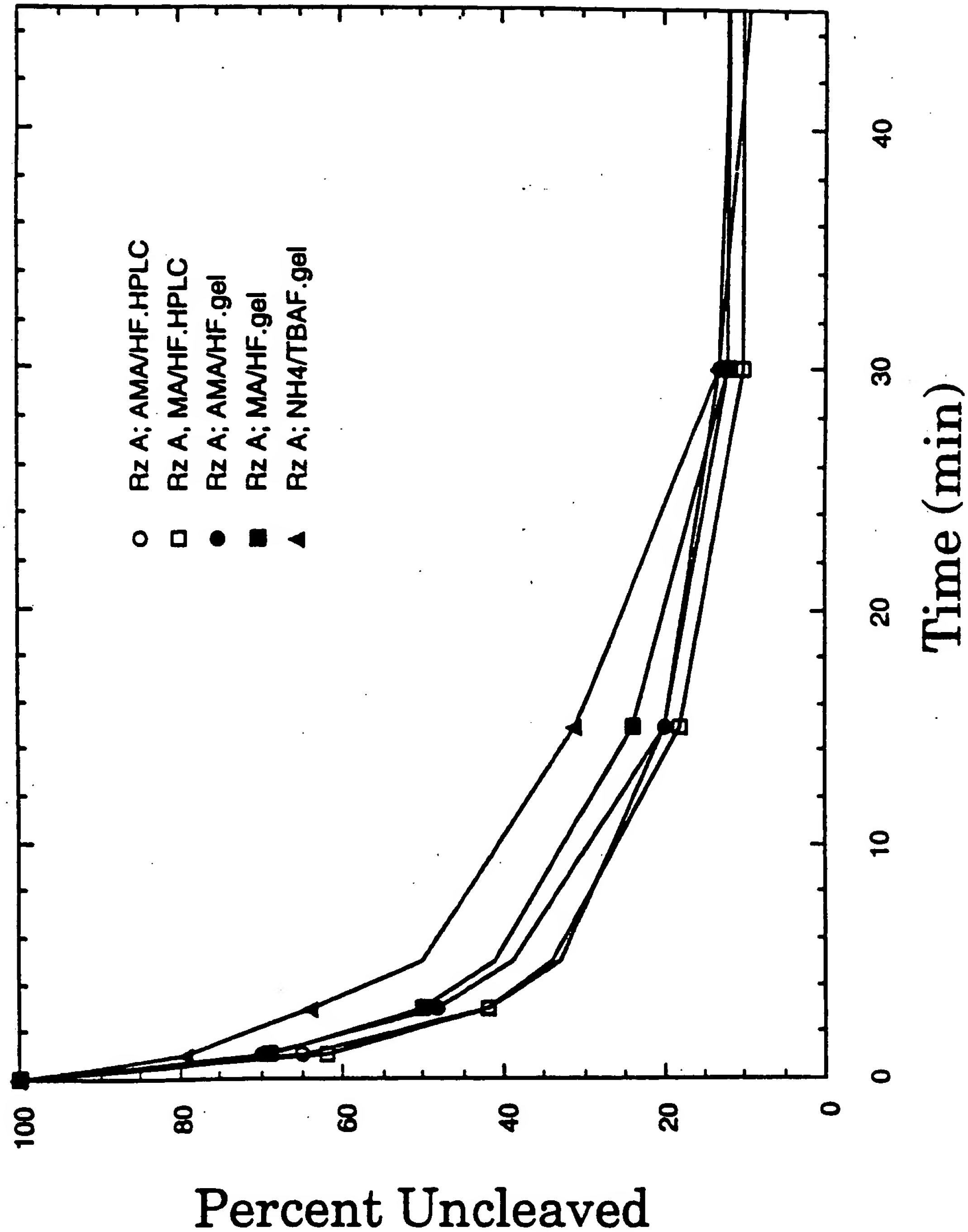
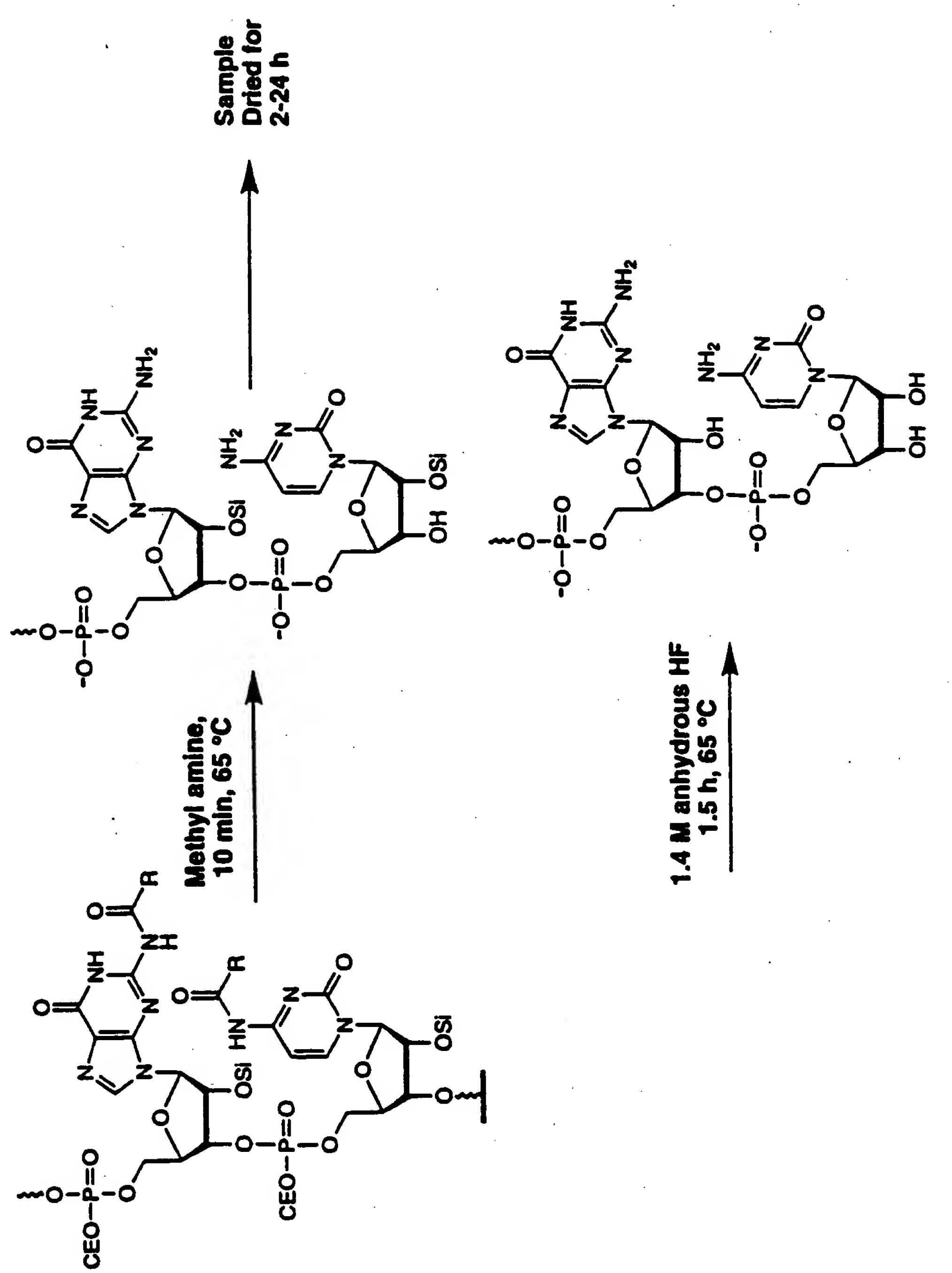
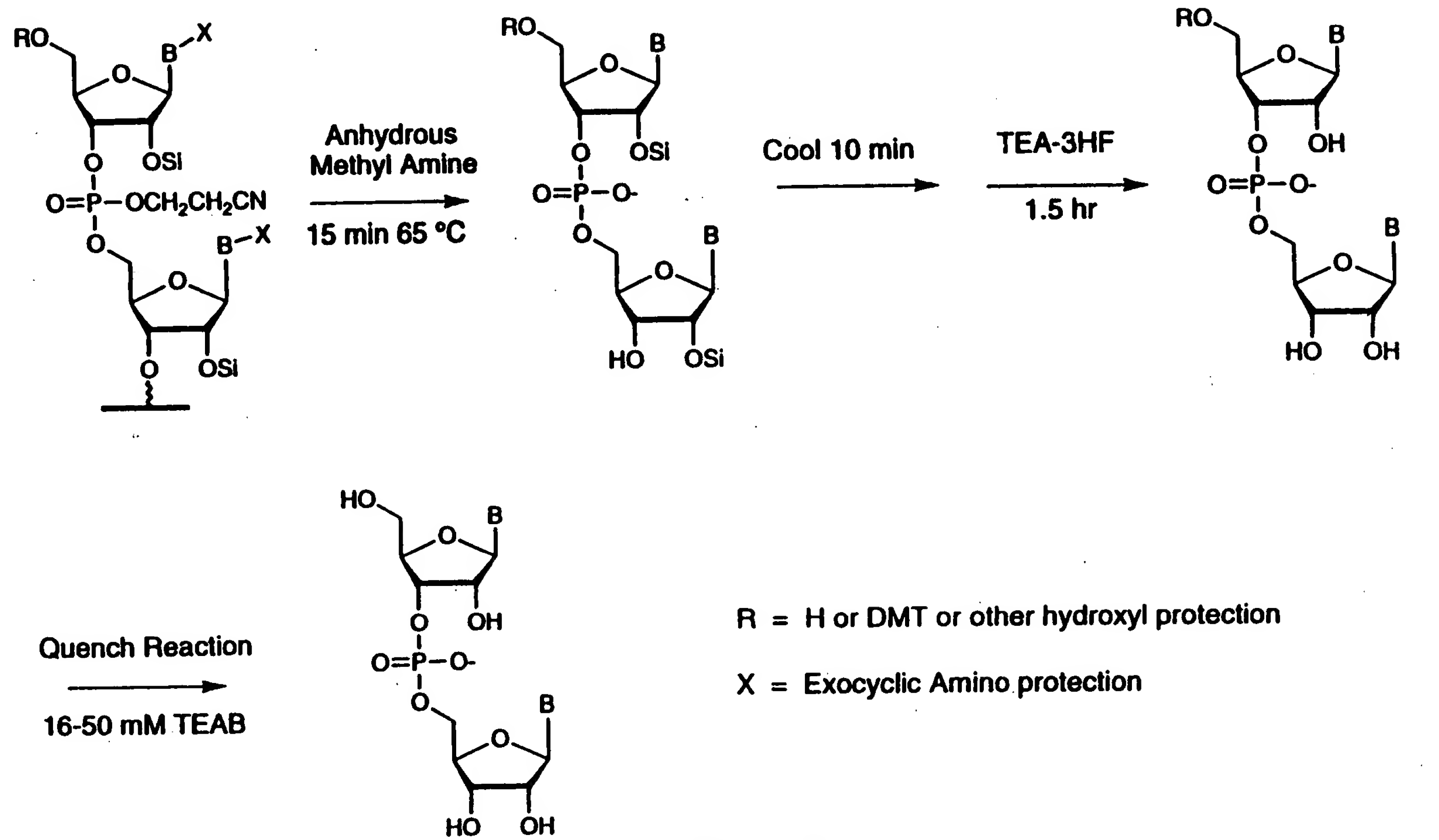

Rz A; AMA/HF.HPLC
Rz A, MA/HF.HPLC
Rz A; AMA/HF.gel
Rz A; MA/HF.gel
Rz A; NH4/TBAF.gel
100
80
60
40
20
0
0
10
20
30
40
Percent Uncleaved
Time (min)

FIG. 11.


Methyl amine, 10 min, 65 °C
Sample Dried for 2-24 h
1.4 M anhydrous HF 1.5 h, 65 °C



R = H or DMT or other hydroxyl protection

X = Exocyclic Amino protection

FIG. 12.

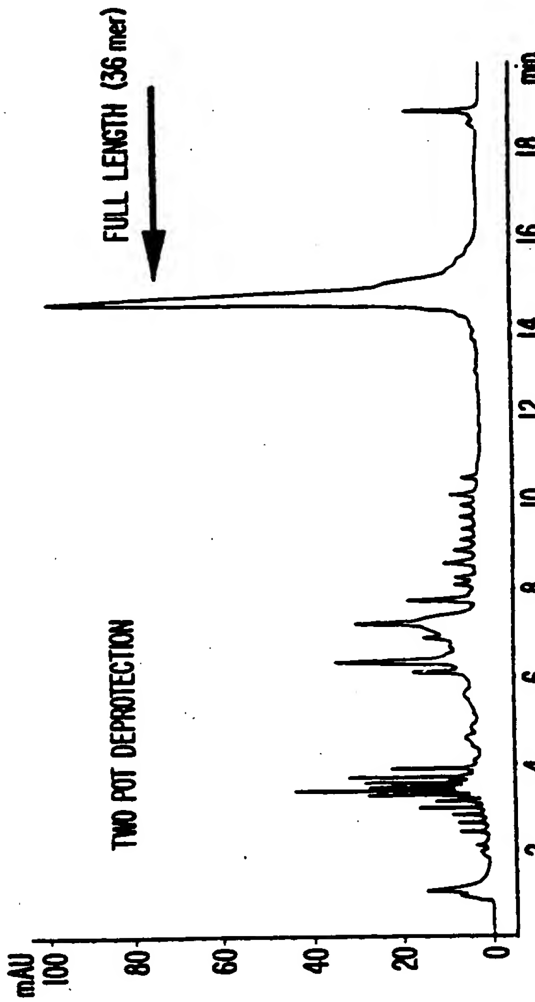


FIG. 13a.

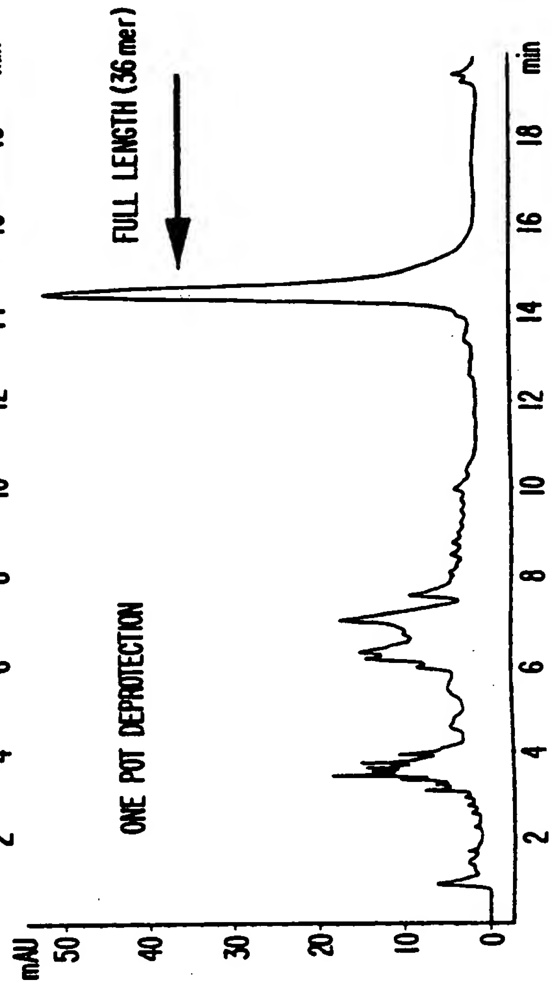


FIG. 13b.

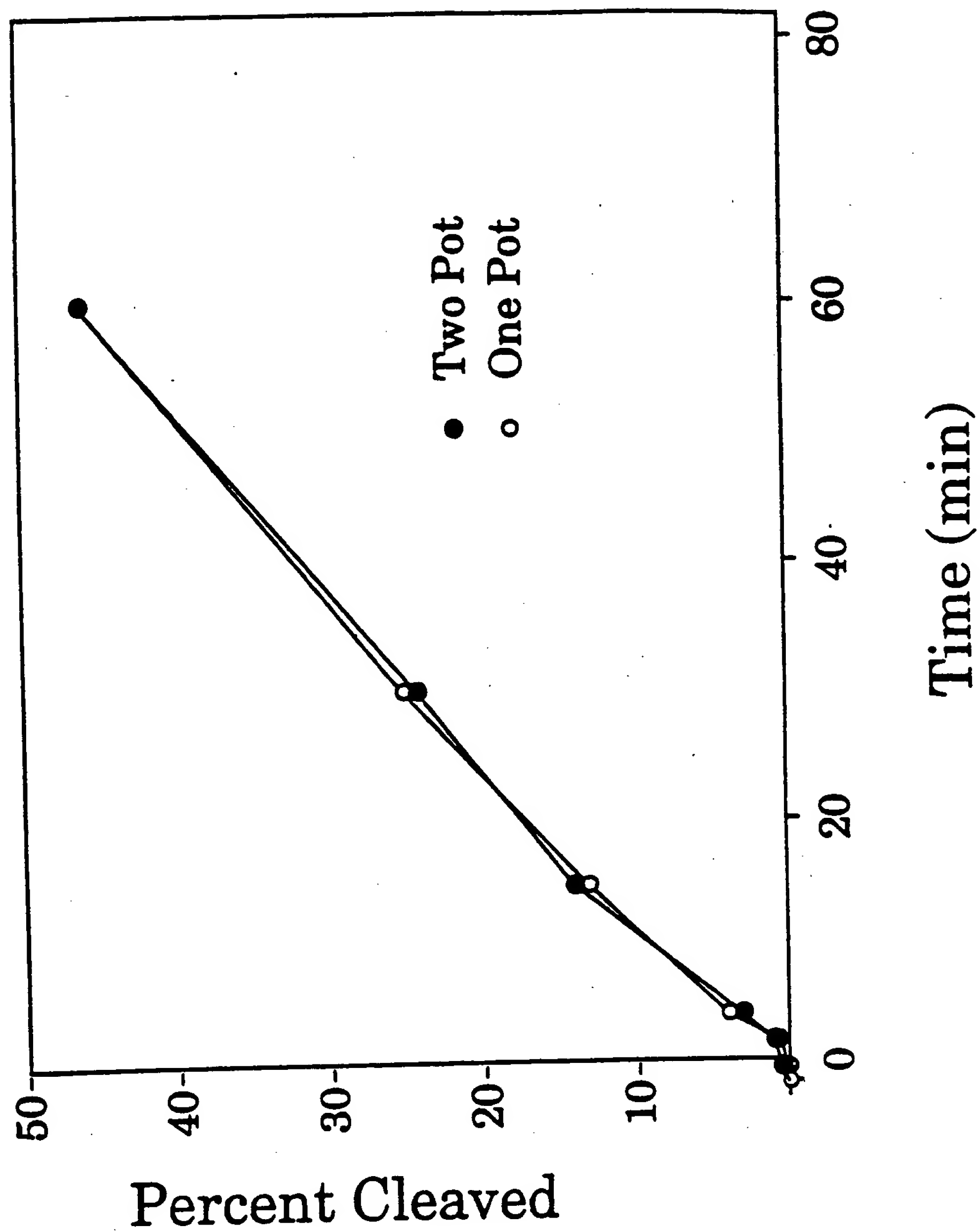
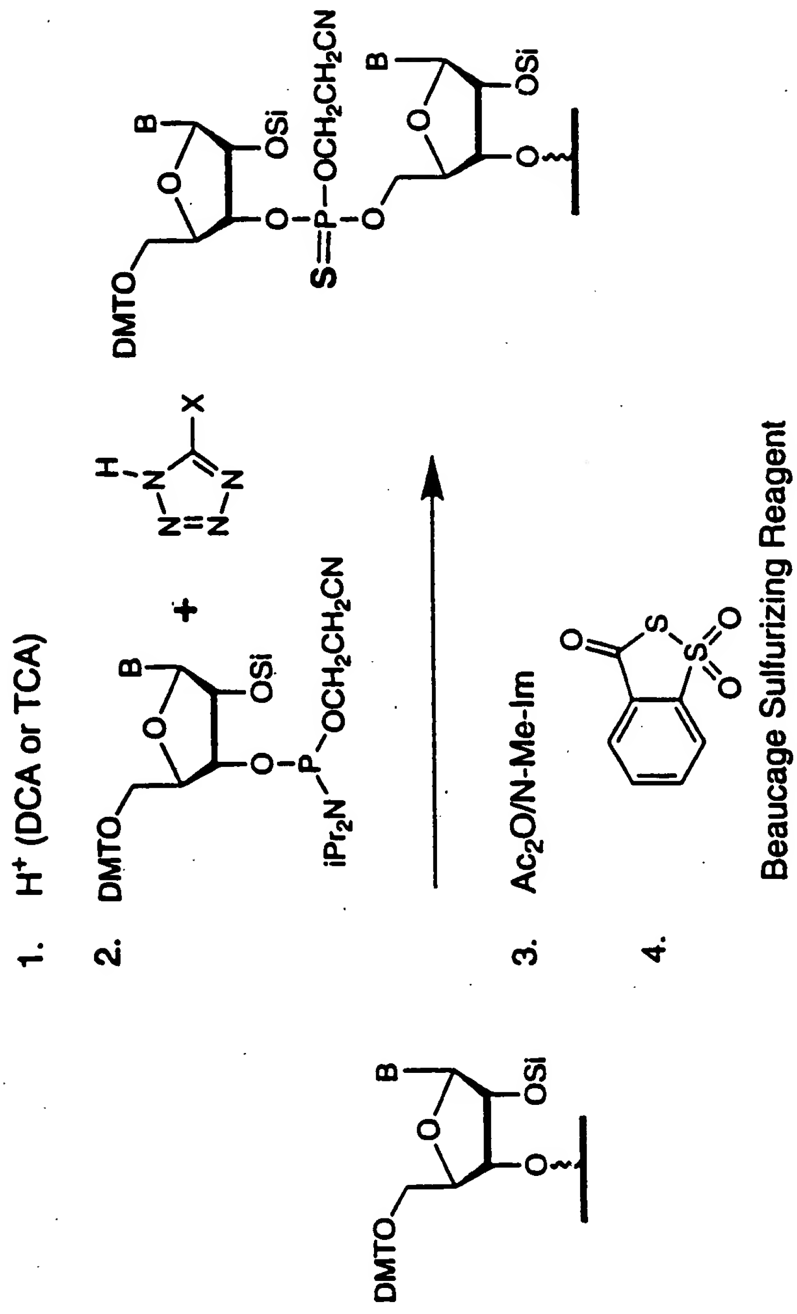

Two Pot
One Pot
Percent Cleaved
0
10
20
30
40
50
Time (min)
0
20
40
60
80

FIG. 14.

FIG. 15.


DMTO
B
O
OSi
O
1. H+ (DCA or TCA)
2. DMTO
B
O
OSi
O
P
iPr2N
OCH2CH2CN
+
H
N
N
N
N
X
3. Ac2O/N-Me-Im
4. Beaucage Sulfurizing Reagent
O
S
S
O
O
DMTO
B
O
OSi
O
S
P
OCH2CH2CN
O
B
O
OSi
O

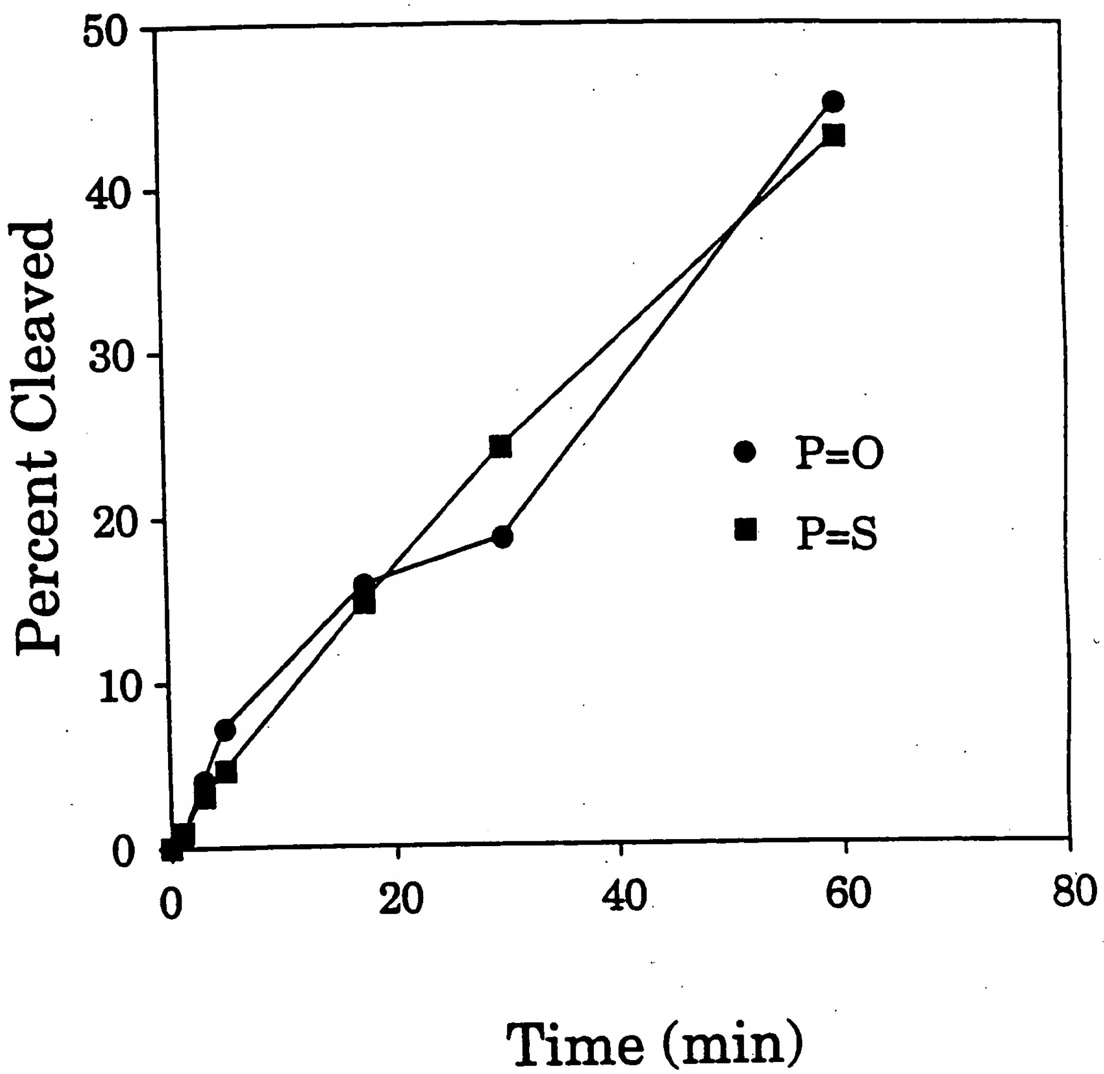
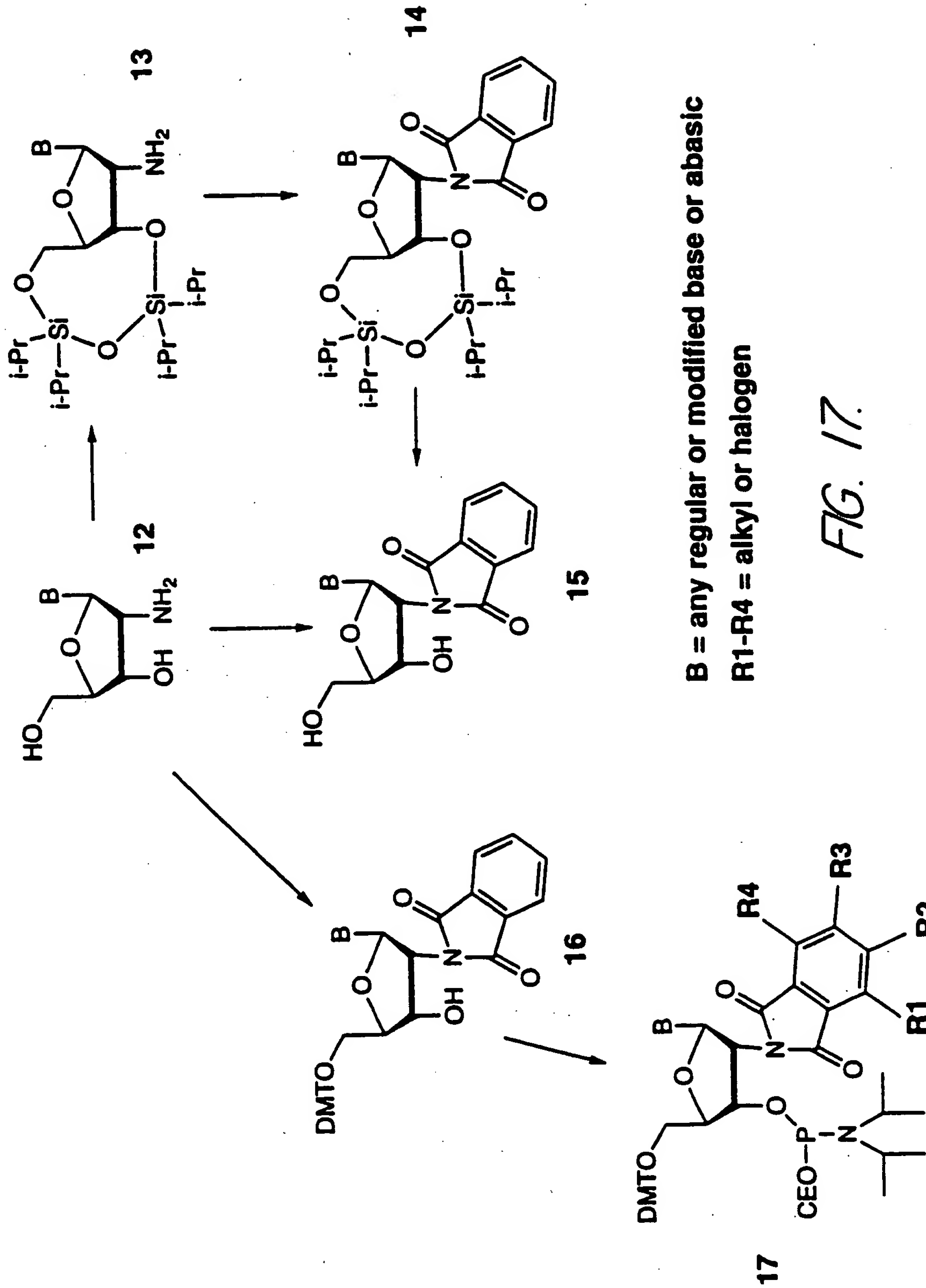


FIG. 16.



B = any regular or modified base or abasic

R1-R4 = alkyl or halogen

FIG. 17.

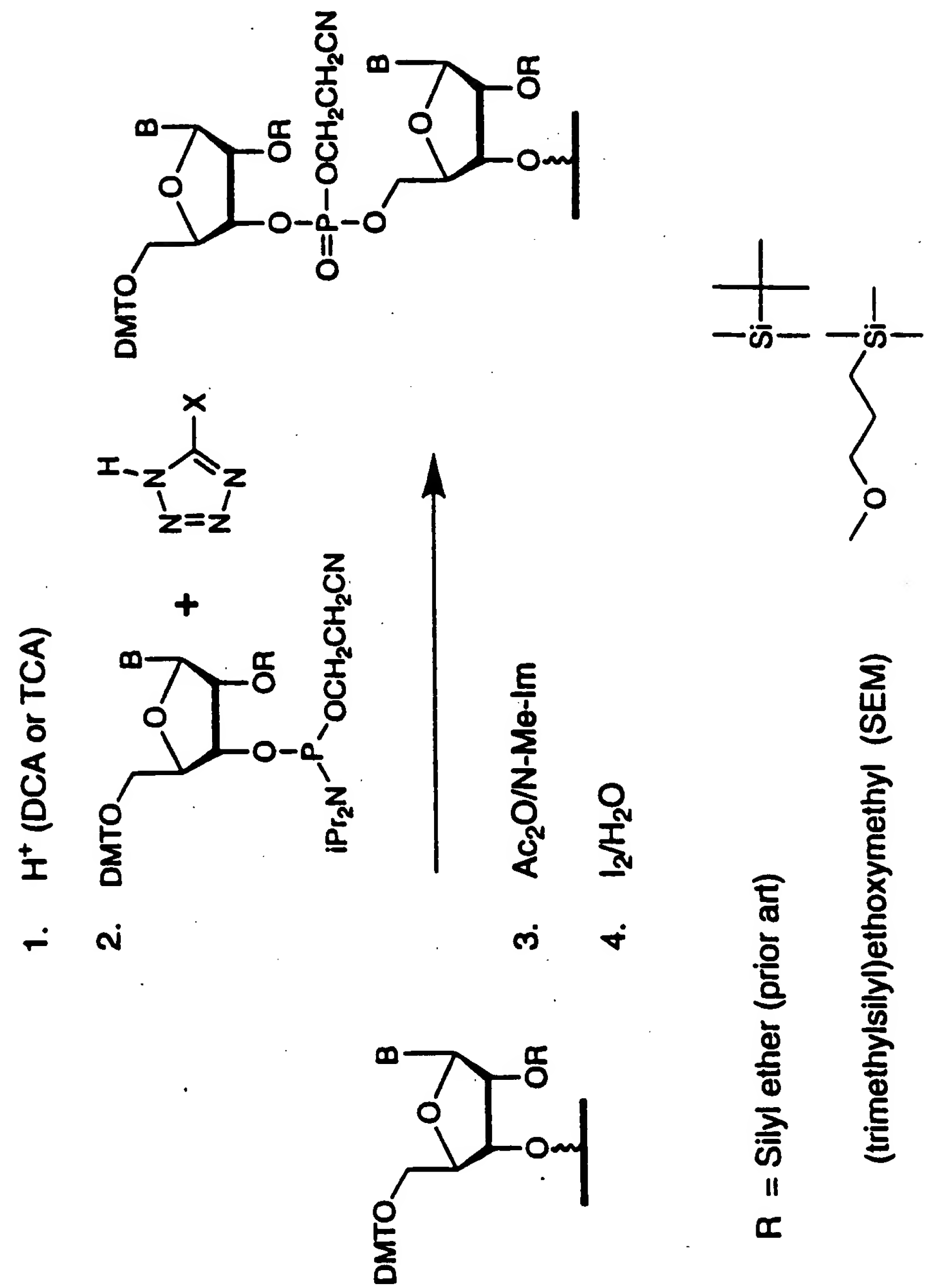
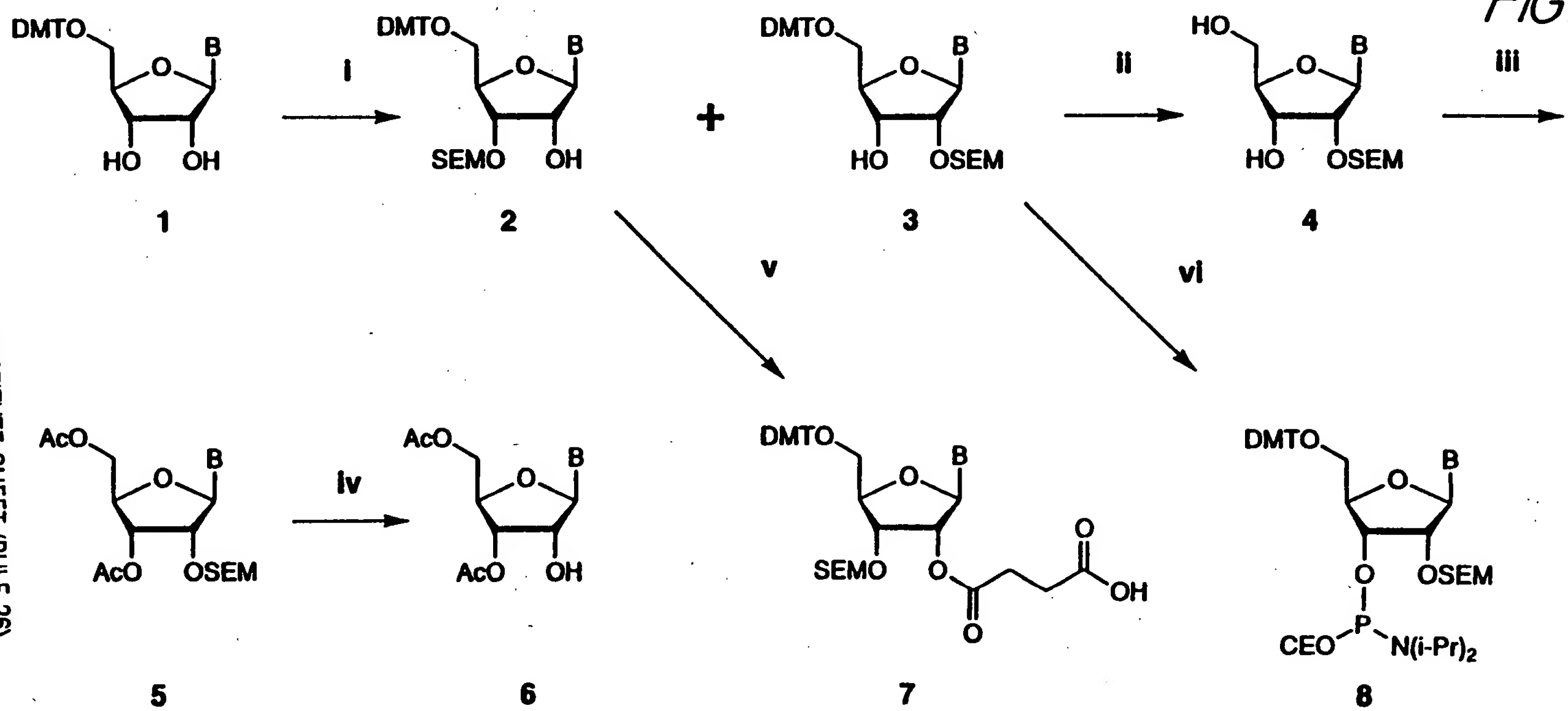

1. H+ (DCA or TCA)
2. DMTO
B
OR
O
iPr2N
P
OCH2CH2CN
+
H
N
N=N
N
X
3. Ac2O/N-Me-Im
4. I2/H2O
DMTO
B
O
OR
O=P-OCH2CH2CN
R = Silyl ether (prior art)
(trimethylsilyl)ethoxymethyl (SEM)
Si

FIG. 18.

FIG. 19.



B = Protected A, C, G, U, T, 2AP, I, DiAP, P *etc.*

SEM = (trimethylsilyl)ethoxymethyl

i) = $SnBu_2O$/SEM-Cl
ii) = H^+
iii) = Ac_2O
iv) = $BF_3{\bullet}OEt_2$
v) = Succinic Anhydride
vi) = P(OCE)(N-iPr_2)Cl

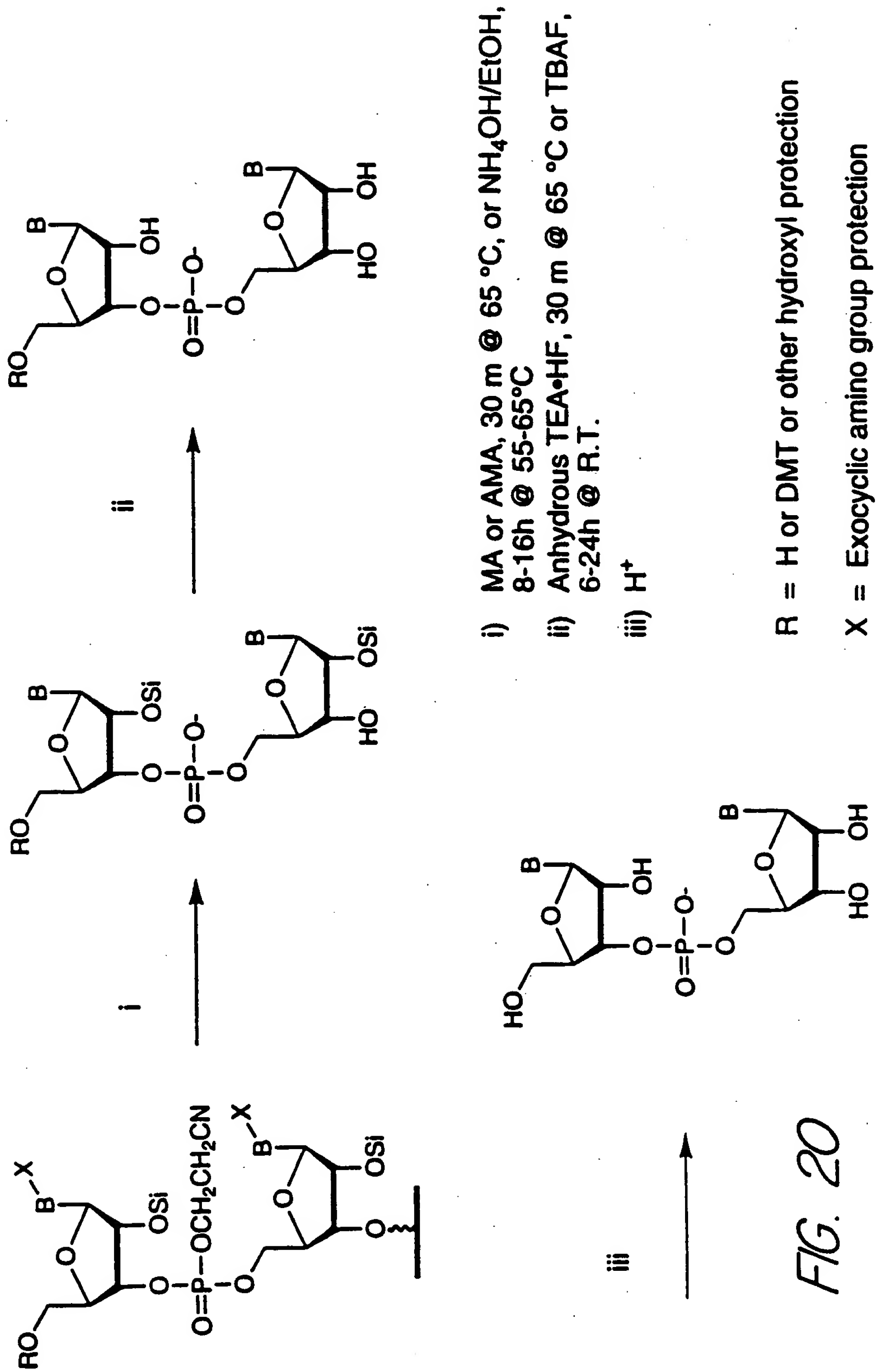
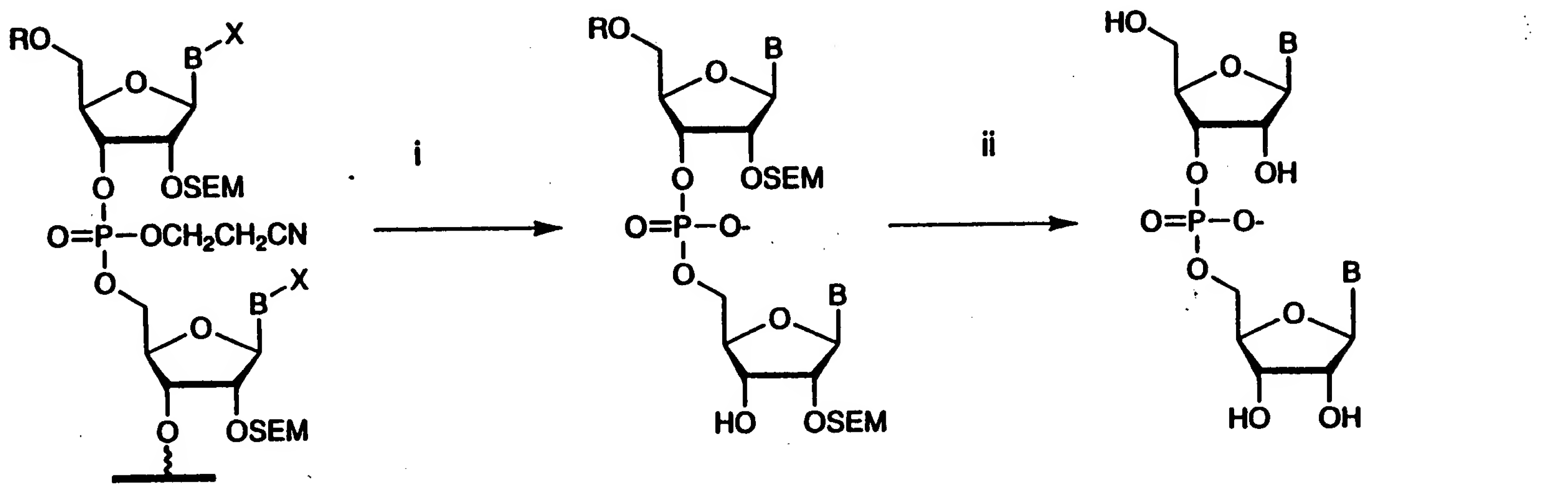

i) MA or AMA, 30 m @ 65 °C, or NH_4OH/EtOH, 8-16h @ 55-65°C
ii) Anhydrous TEA•HF, 30 m @ 65 °C or TBAF, 6-24h @ R.T.
iii) H^+
R = H or DMT or other hydroxyl protection
X = Exocyclic amino group protection

FIG. 20

FIG. 21.



i) MA or AMA, 30 m @ 65 °C or NH_4OH or NH_4OH/EtOH, 8-16h @ 55-65°C

ii) $BF_3{\bullet}OEt_2$

SEM = (trimethylsilyl)ethoxymethyl

R = H or DMT or other hydroxyl protection

X = Exocyclic amino group protection

FIG. 22.

2'-O-SEM PROTECTED U 10-mer
DEPROTECTED WITH $BF_3 \cdot OEt_2$
30m, 3eq./nucleotide

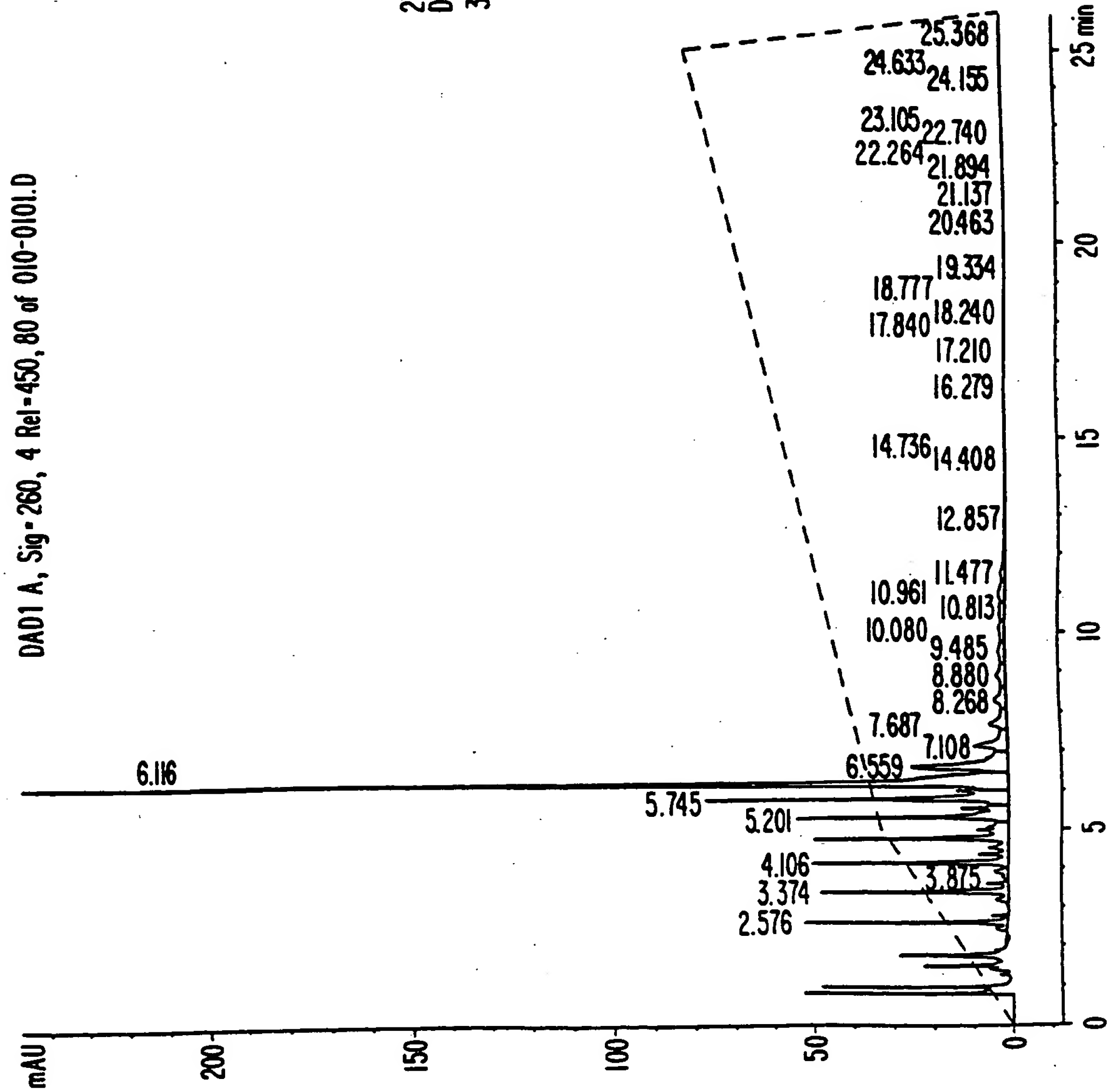
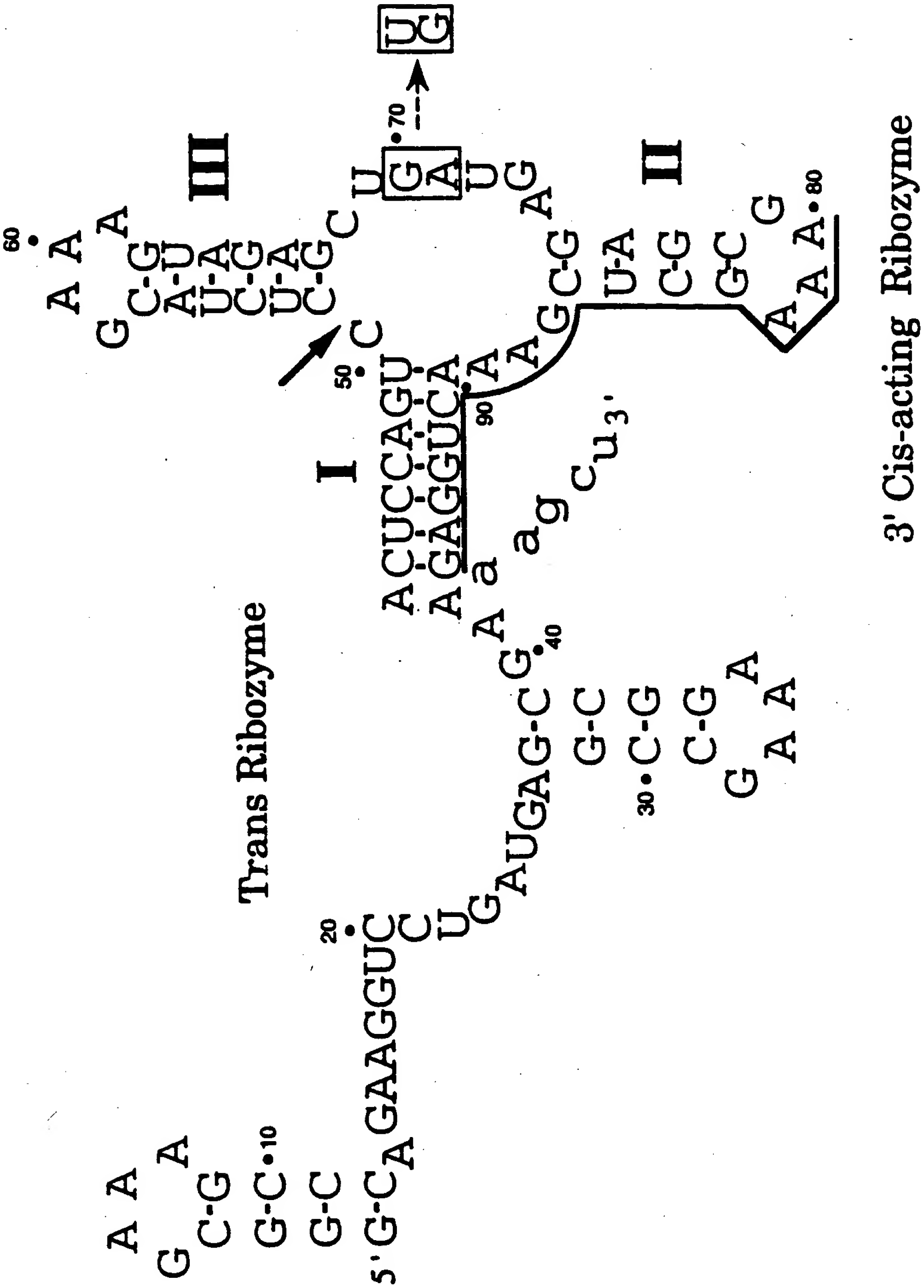


FIG. 23.



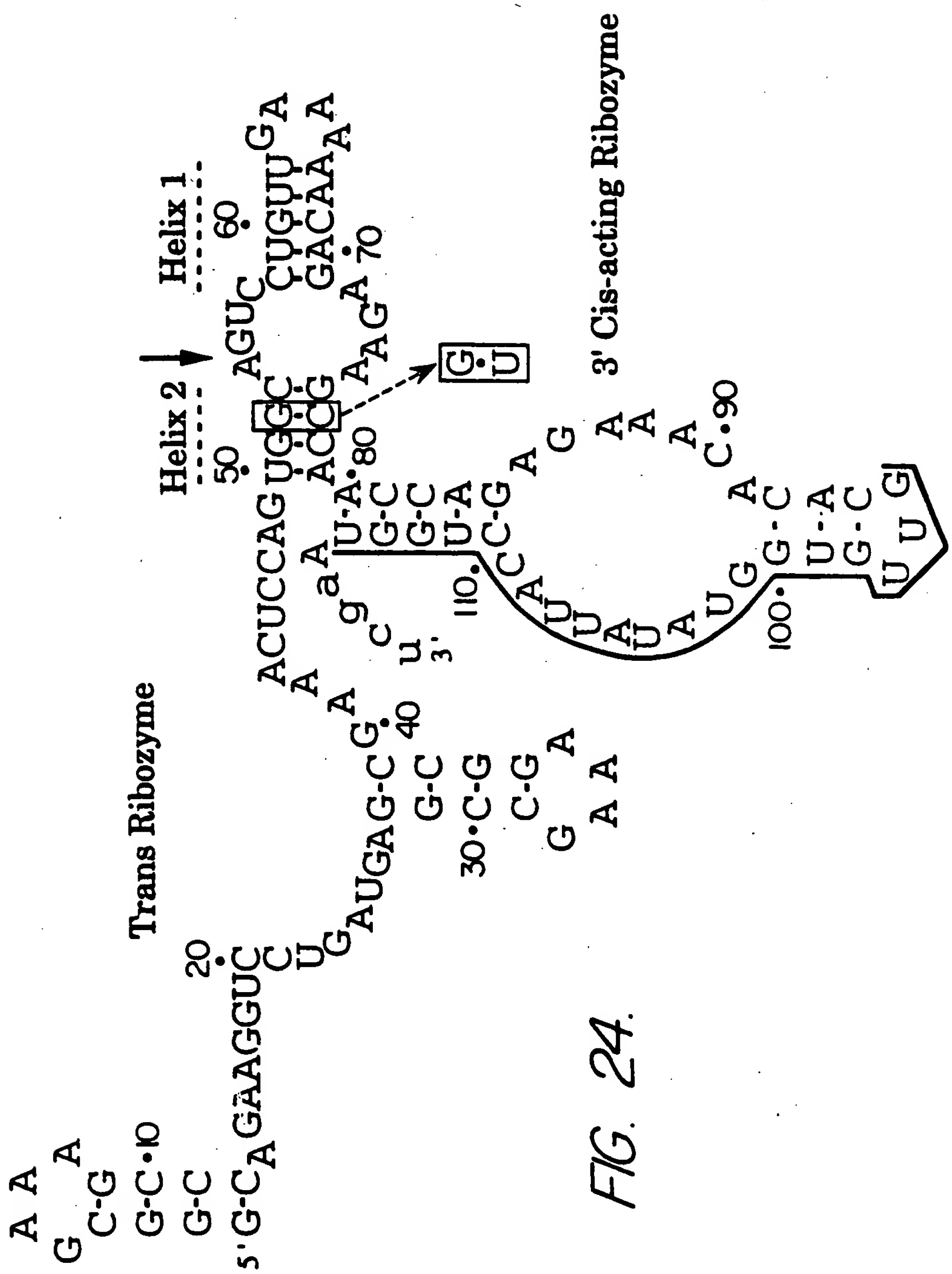


FIG. 24.

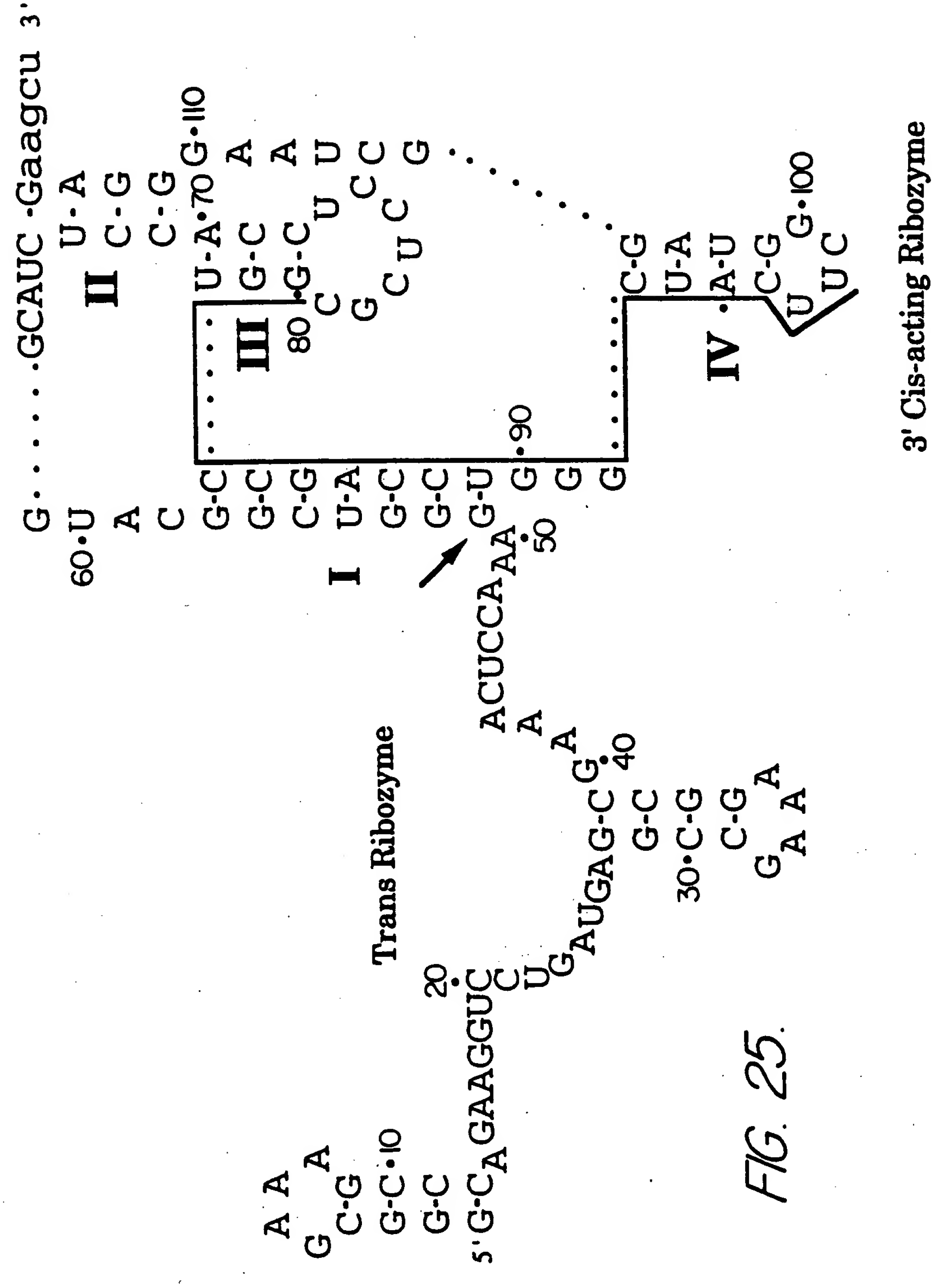


FIG. 25.

FIG. 26.

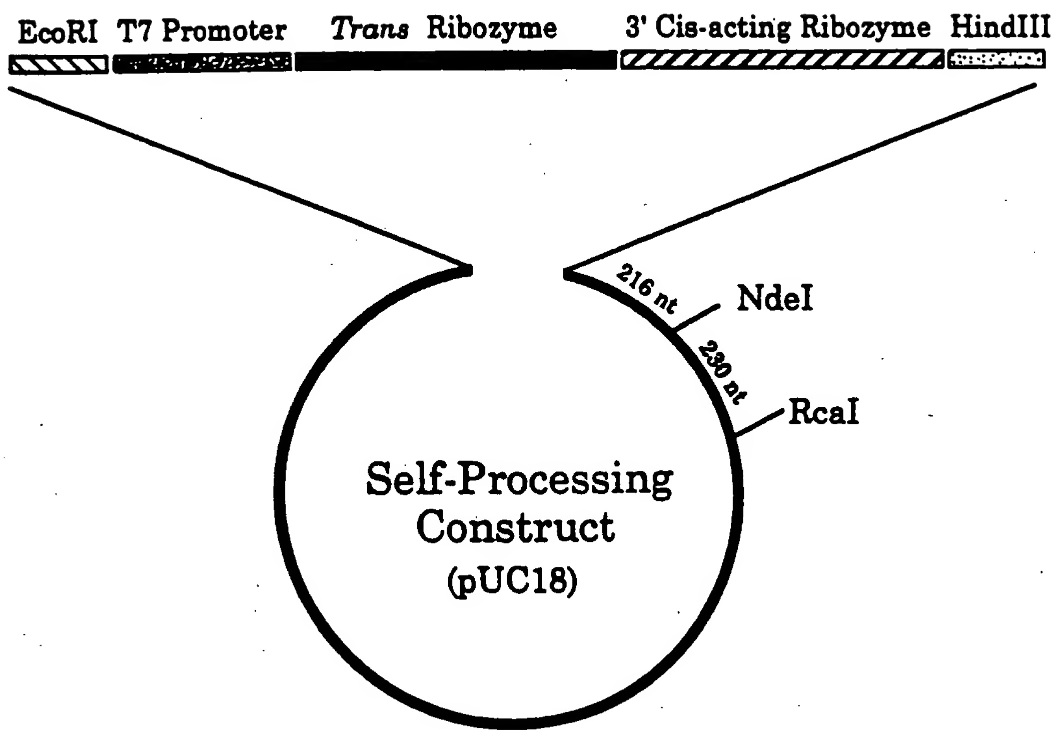
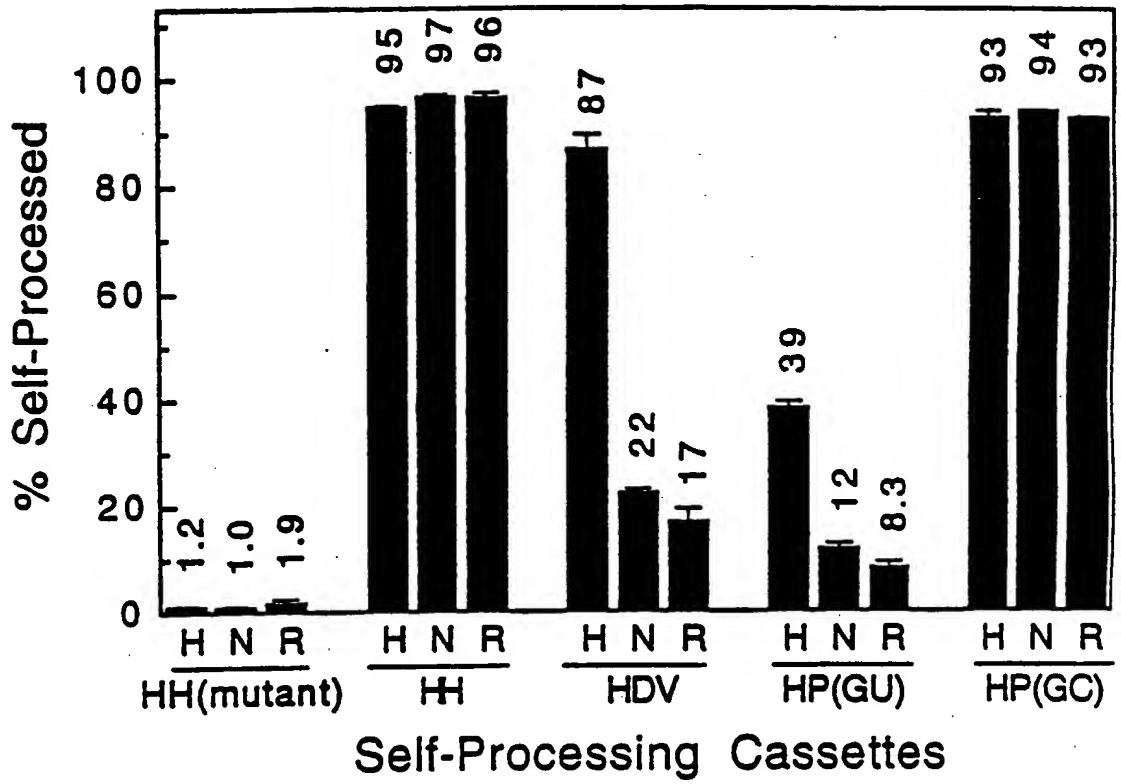

EcoRI
T7 Promoter
Trans Ribozyme
3' Cis-acting Ribozyme
HindIII
216 nt
NdeI
230 nt
RcaI
Self-Processing
Construct
(pUC18)

FIG. 27.


% Self-Processed
100
80
60
40
20
0
1.2
1.0
1.9
95
97
96
87
22
17
39
12
8.3
93
94
93
H N R
H N R
H N R
H N R
H N R
HH(mutant)
HH
HDV
HP(GU)
HP(GC)
Self-Processing Cassettes

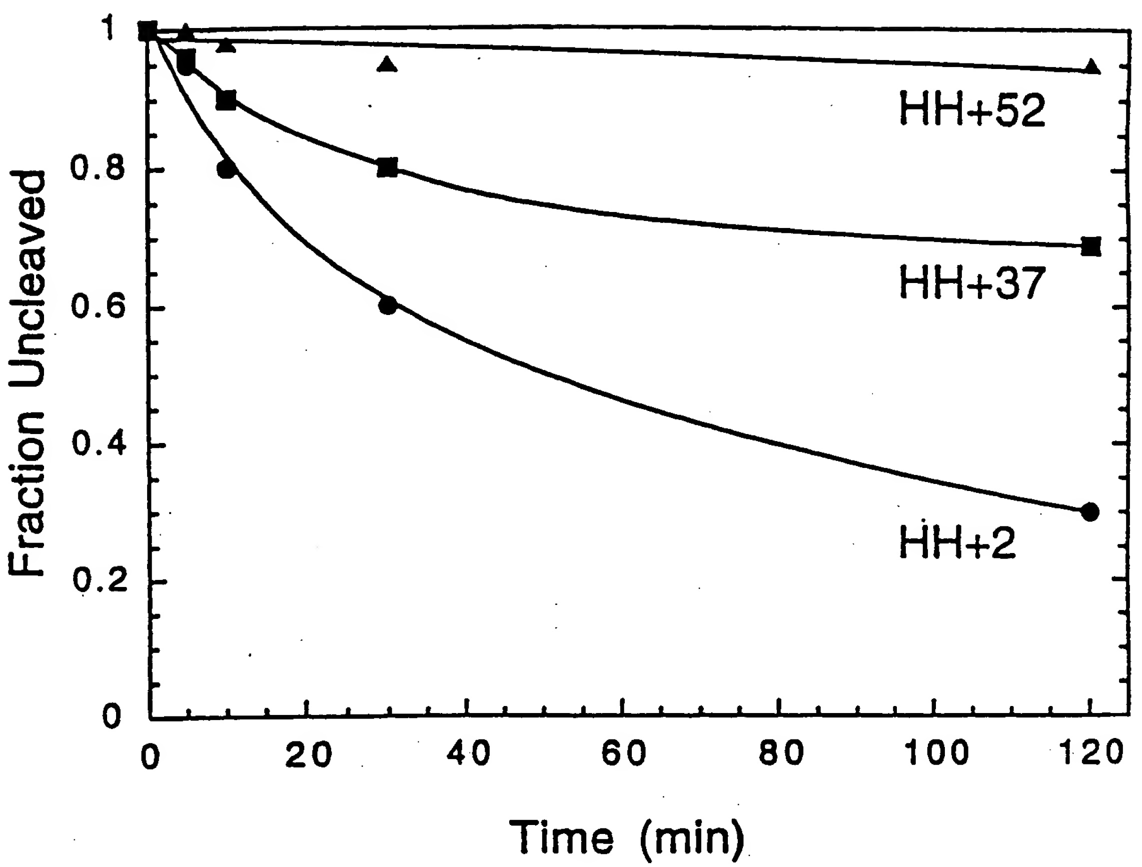
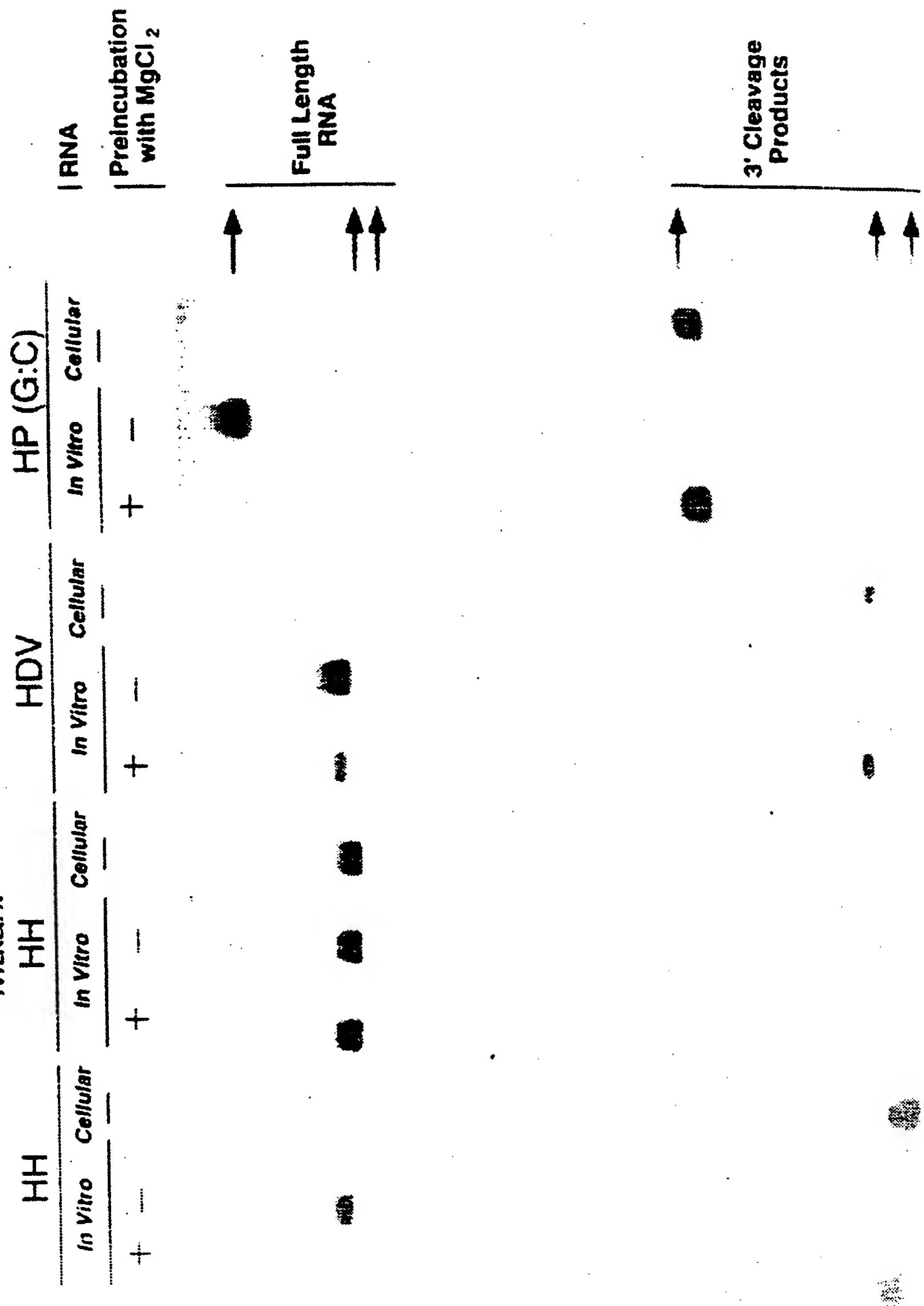

1
0.8
0.6
0.4
0.2
0
Fraction Uncleaved
0
20
40
60
80
100
120
Time (min)
HH+52
HH+37
HH+2

FIG. 28.

FIG. 29.


HH
Mutant HH
HDV
HP (G:C)
In Vitro
Cellular
RNA
Preincubation with $MgCl_2$
+
-
Full Length RNA
3' Cleavage Products

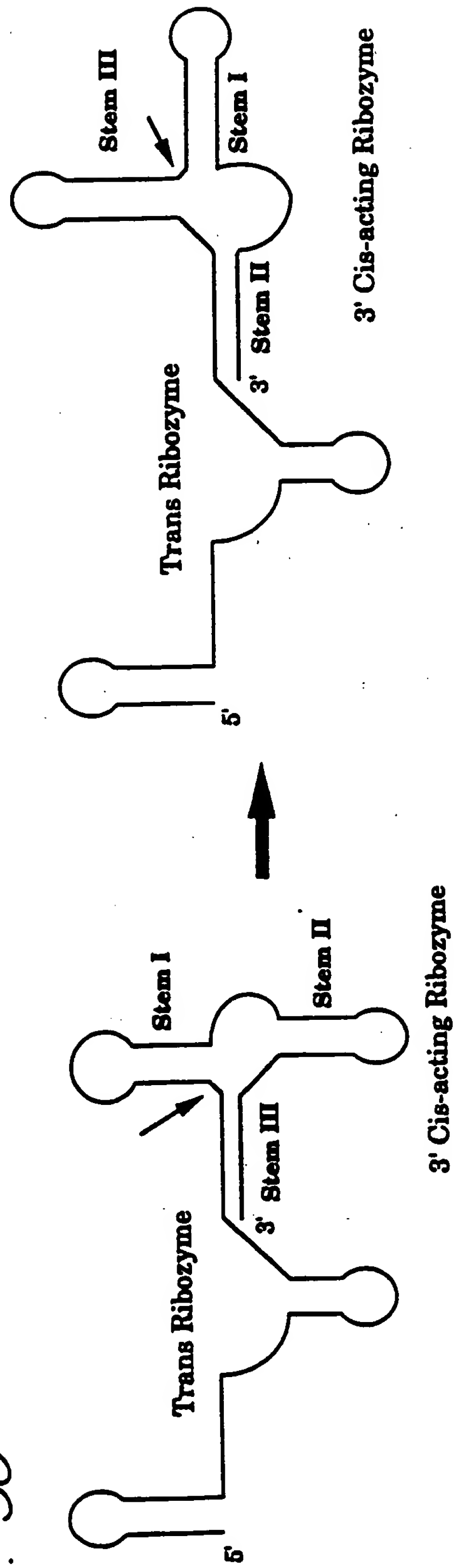

5'
Trans Ribozyme
3'
Stem III
Stem I
Stem II
3' Cis-acting Ribozyme
5'
Trans Ribozyme
3'
Stem II
Stem III
Stem I
3' Cis-acting Ribozyme

FIG. 30

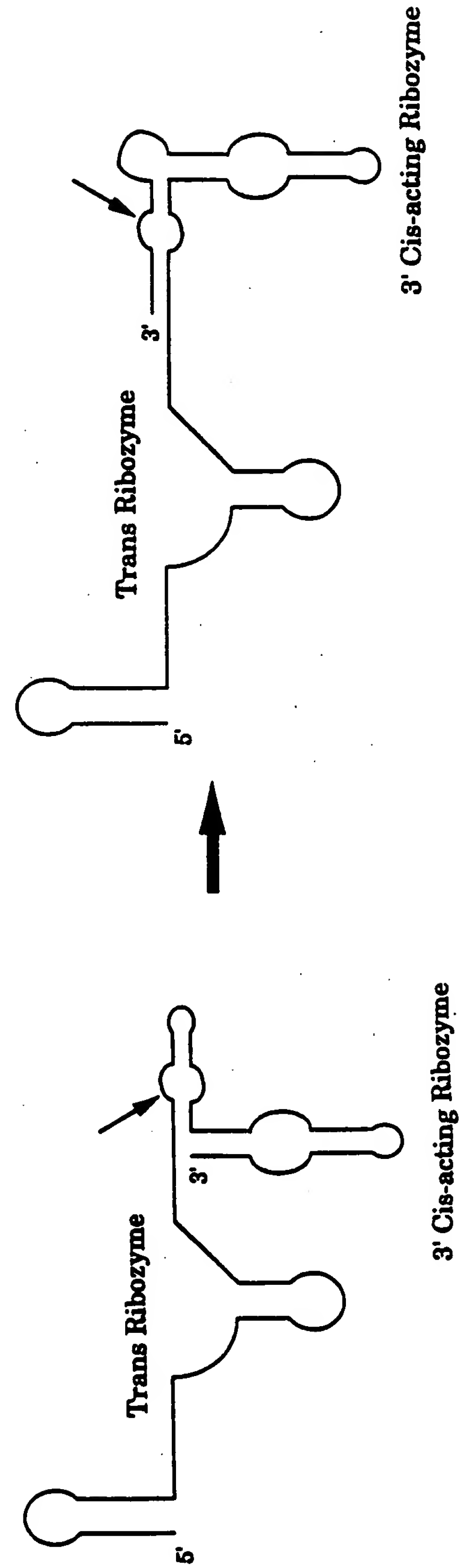
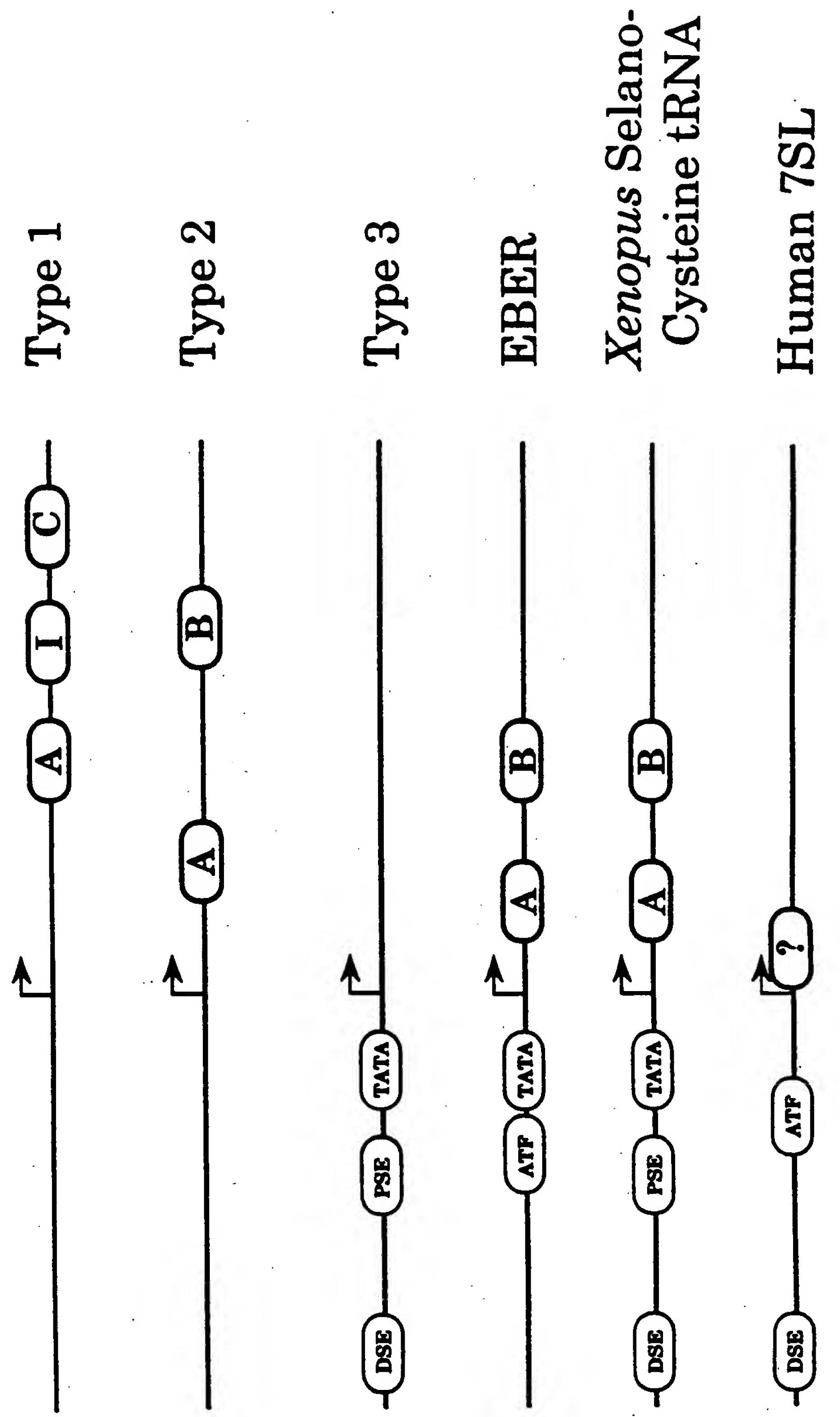

5'
Trans Ribozyme
3'
3' Cis-acting Ribozyme
5'
Trans Ribozyme
3'
3' Cis-acting Ribozyme

FIG. 31.

FIG. 32.


Type 1
Type 2
Type 3
EBER
Xenopus Selano-Cysteine tRNA
Human 7SL
A
I
C
B
?
TATA
PSE
ATF
DSE

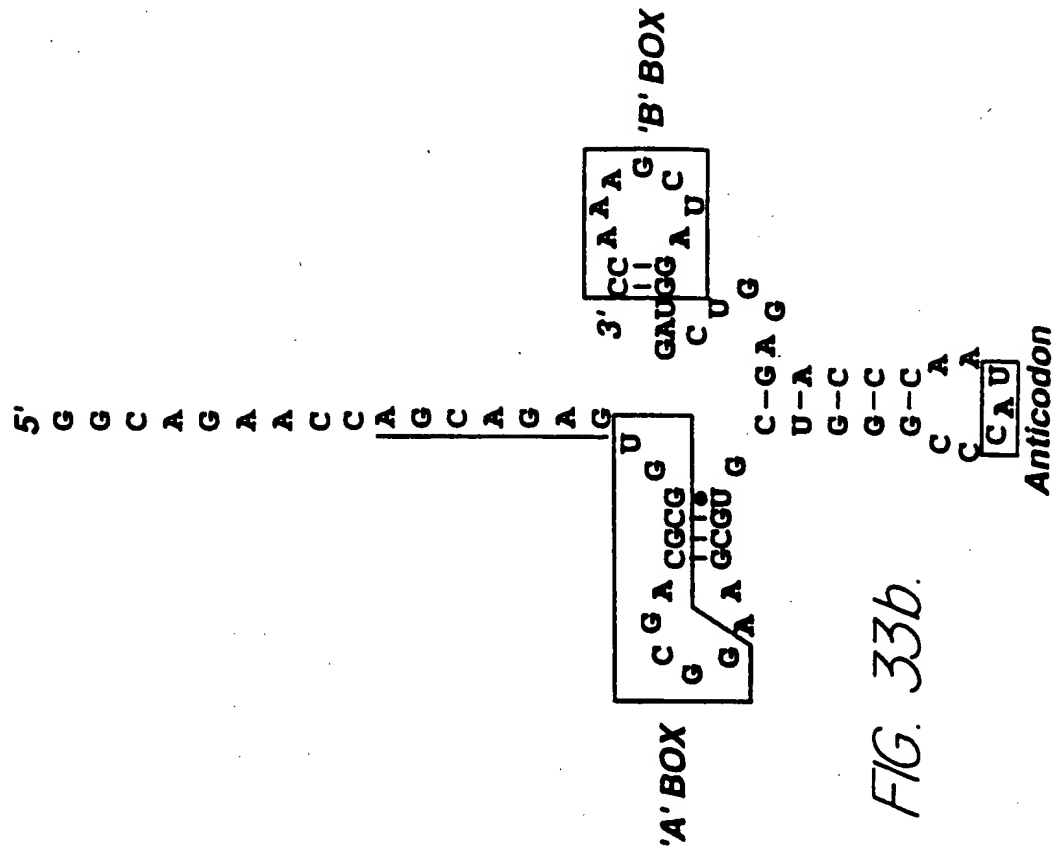


FIG. 33b.

Δ 3-5

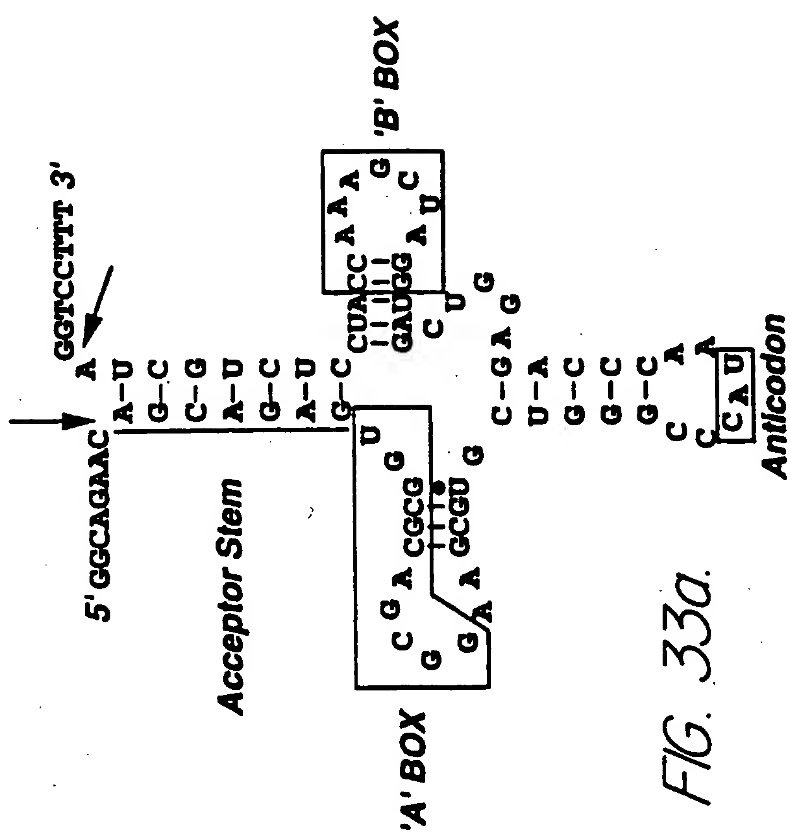


FIG. 33a.

$tRNA_i^{met}$

FIG. 34a.

Δ3-5/HHI

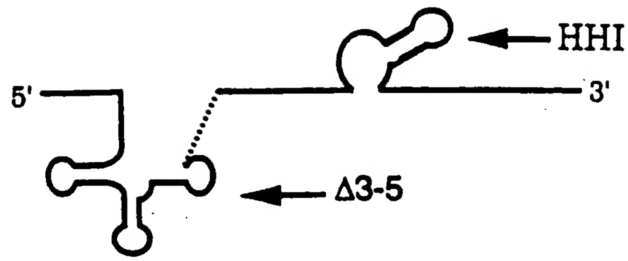


FIG. 34b.

S3

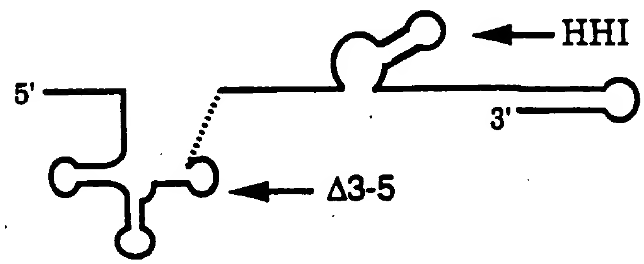


FIG. 34c.

S5

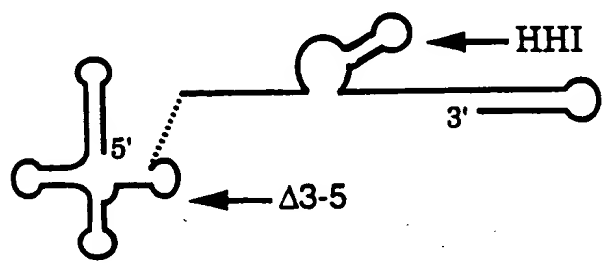


FIG. 34d.

S35

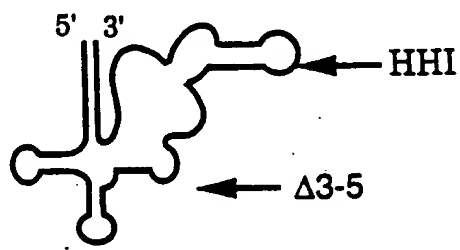
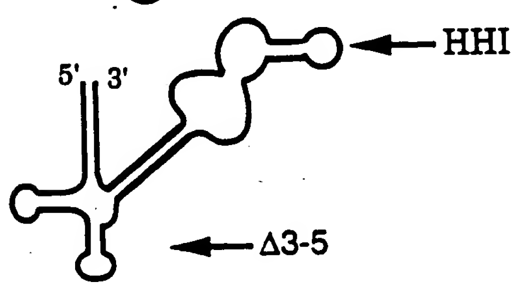


FIG. 34e.

S35Plus



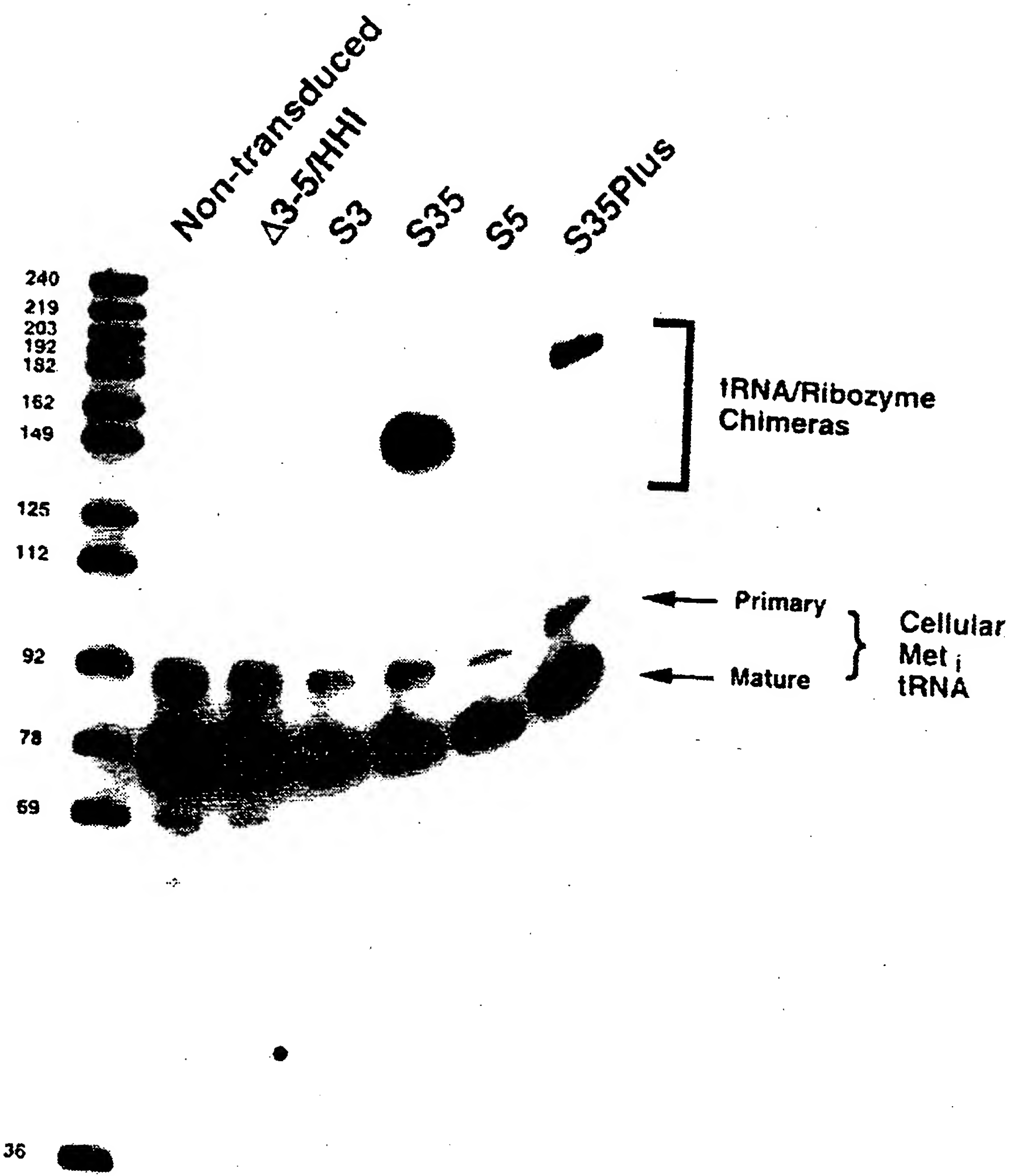

Non-transduced
Δ3-5/HHI
S3
S35
S5
S35Plus
240
219
203
192
182
162
149
125
112
92
78
69
36
tRNA/Ribozyme Chimeras
Primary
Mature
Cellular Met i tRNA

FIG. 35.

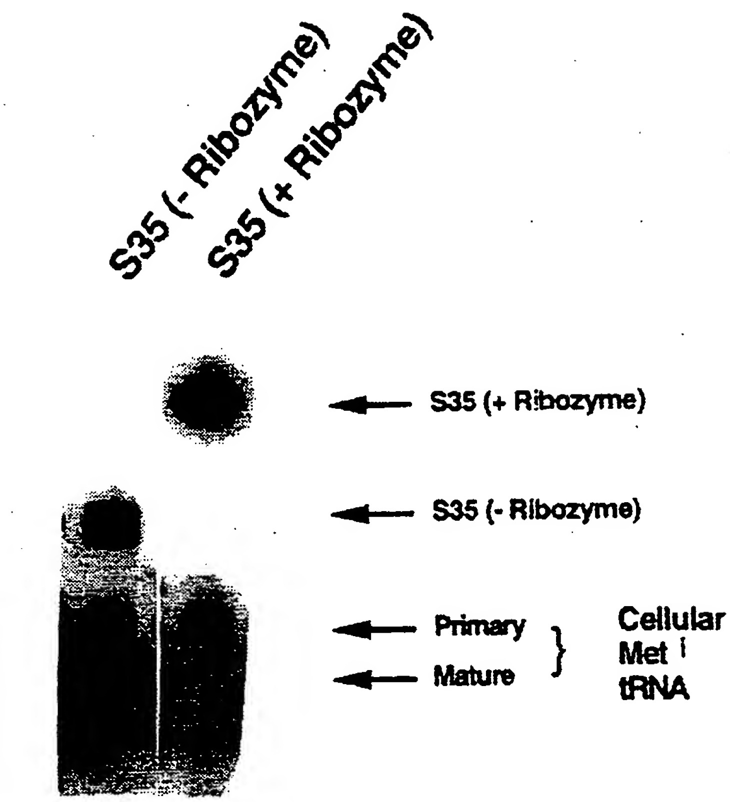

S35 (- Ribozyme)
S35 (+ Ribozyme)
S35 (+ Ribozyme)
S35 (- Ribozyme)
Primary
Mature
Cellular
Met i
tRNA

FIG. 36.

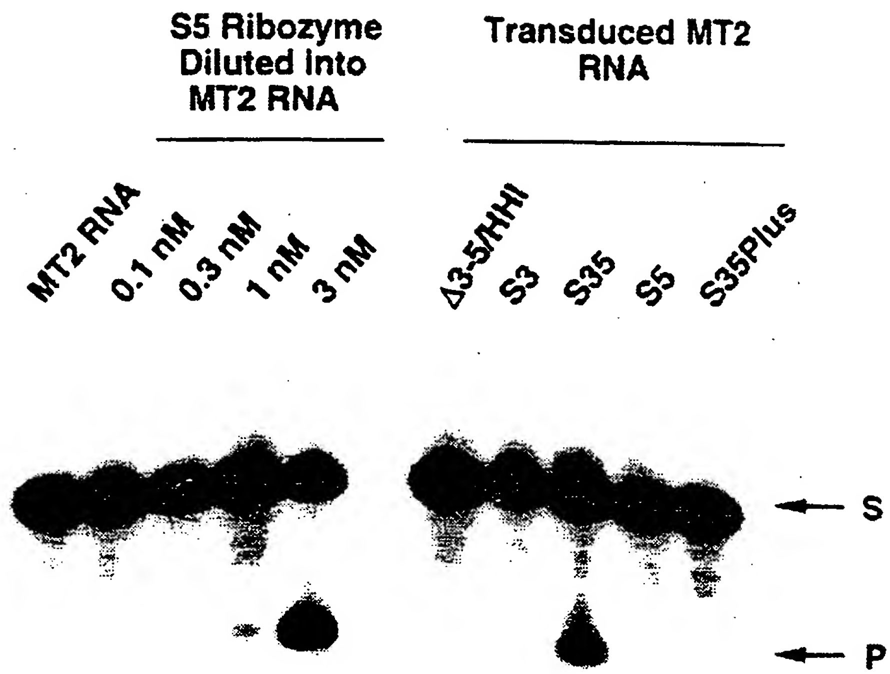
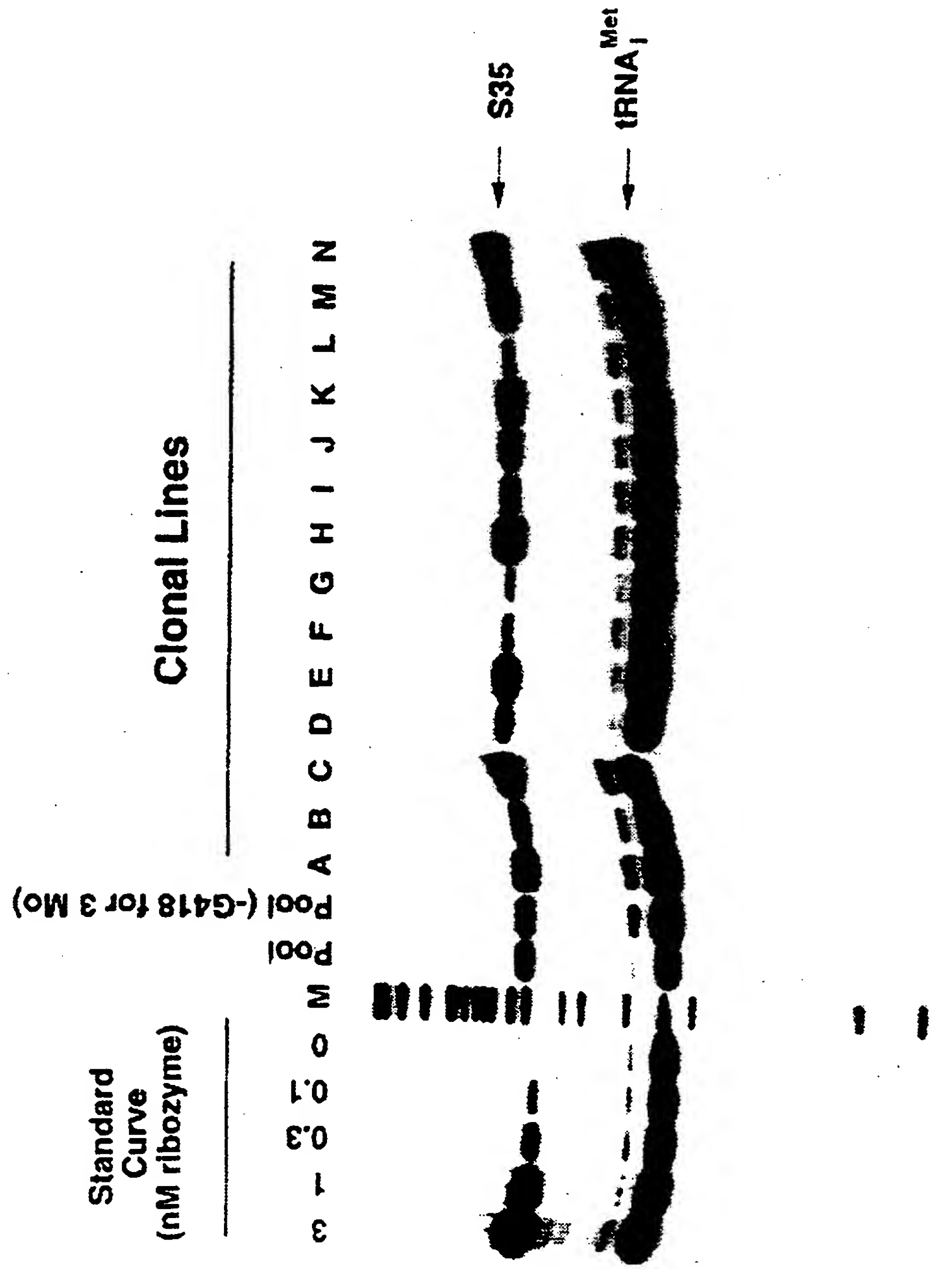

S5 Ribozyme Diluted Into MT2 RNA
Transduced MT2 RNA
MT2 RNA
0.1 nM
0.3 nM
1 nM
3 nM
Δ3-5/HHI
S3
S35
S5
S35Plus
S
P

FIG. 37.

FIG. 38


Standard Curve (nM ribozyme)
3 1 0.3 0.1 0
M
Pool
Pool (-G418 for 3 Mo)
Clonal Lines
A B C D E F G H I J K L M N
S35
tRNA$_i^{Met}$

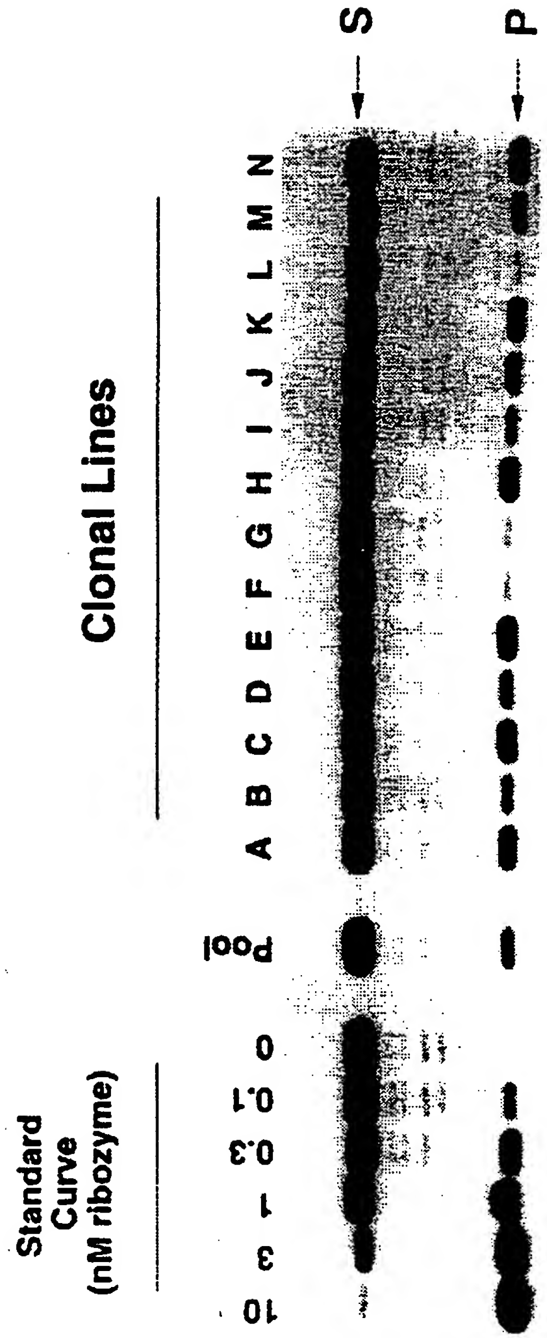

Standard
Curve
(nM ribozyme)
10 3 1 0.3 0.1 0
Pool
Clonal Lines
A B C D E F G H I J K L M N
S
P

FIG. 39.

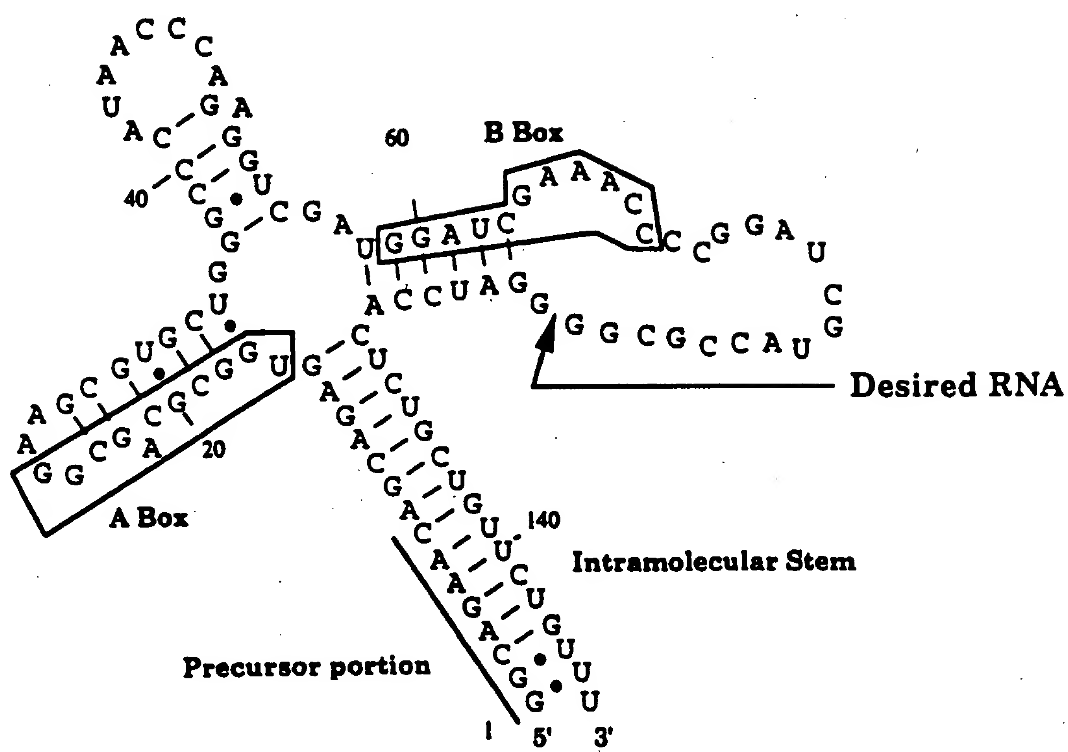

B Box
A Box
Desired RNA
Intramolecular Stem
Precursor portion
60
40
20
140
1
5'
3'

FIG. 40.

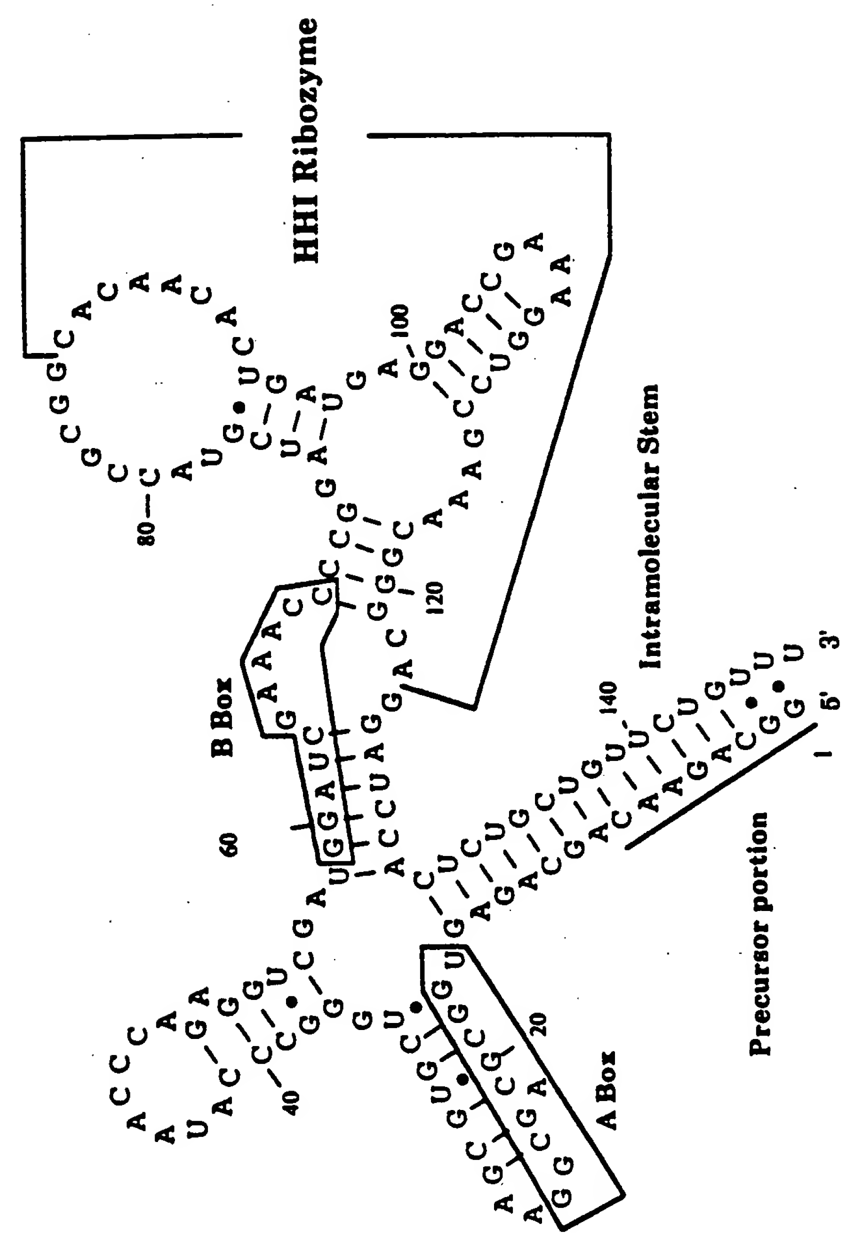

HHI Ribozyme
B Box
A Box
Intramolecular Stem
Precursor portion
5'
3'
20
40
60
80
100
120
140

FIG. 41.

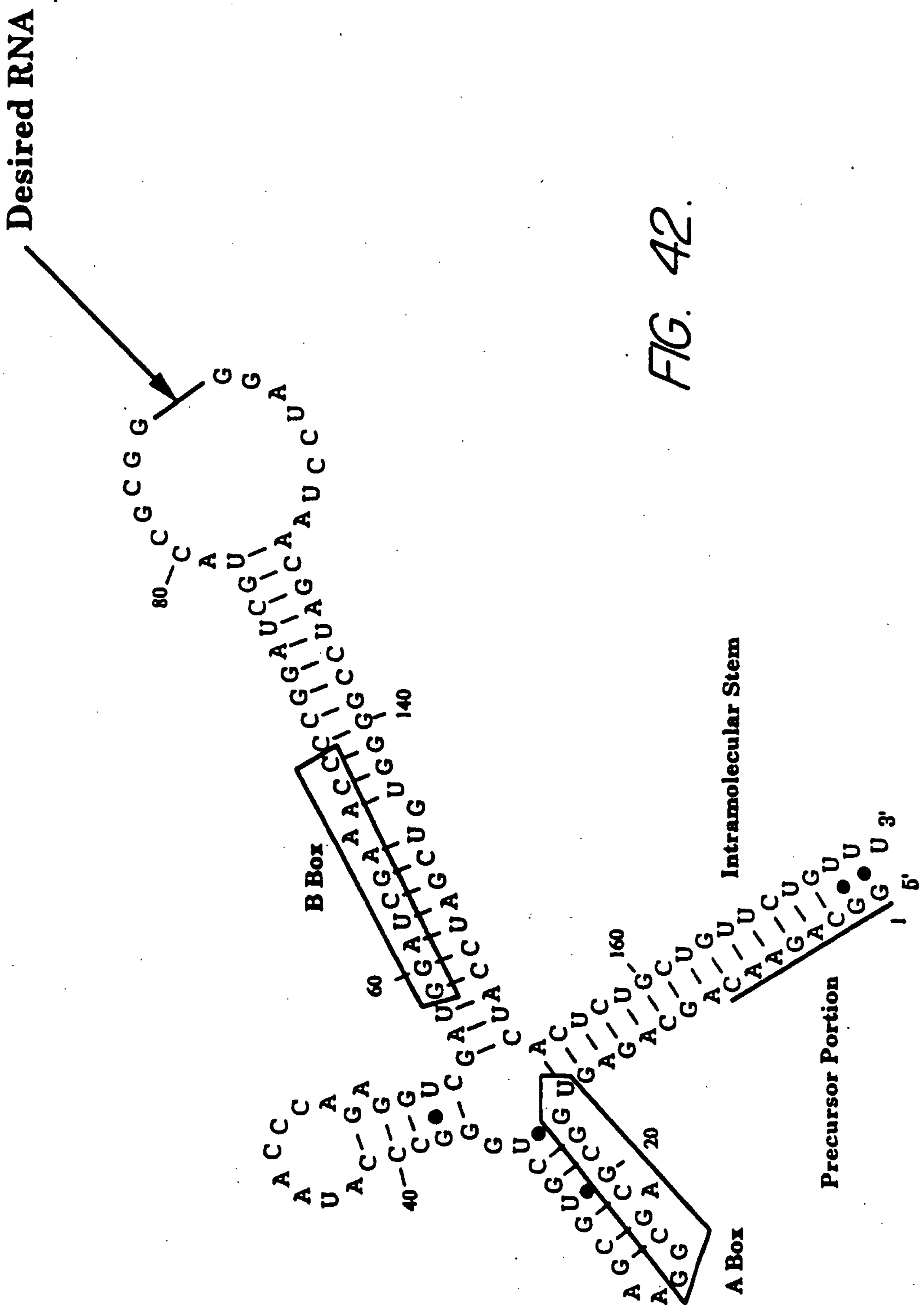

Desired RNA
B Box
A Box
Intramolecular Stem
Precursor Portion
5'
3'

FIG. 42.

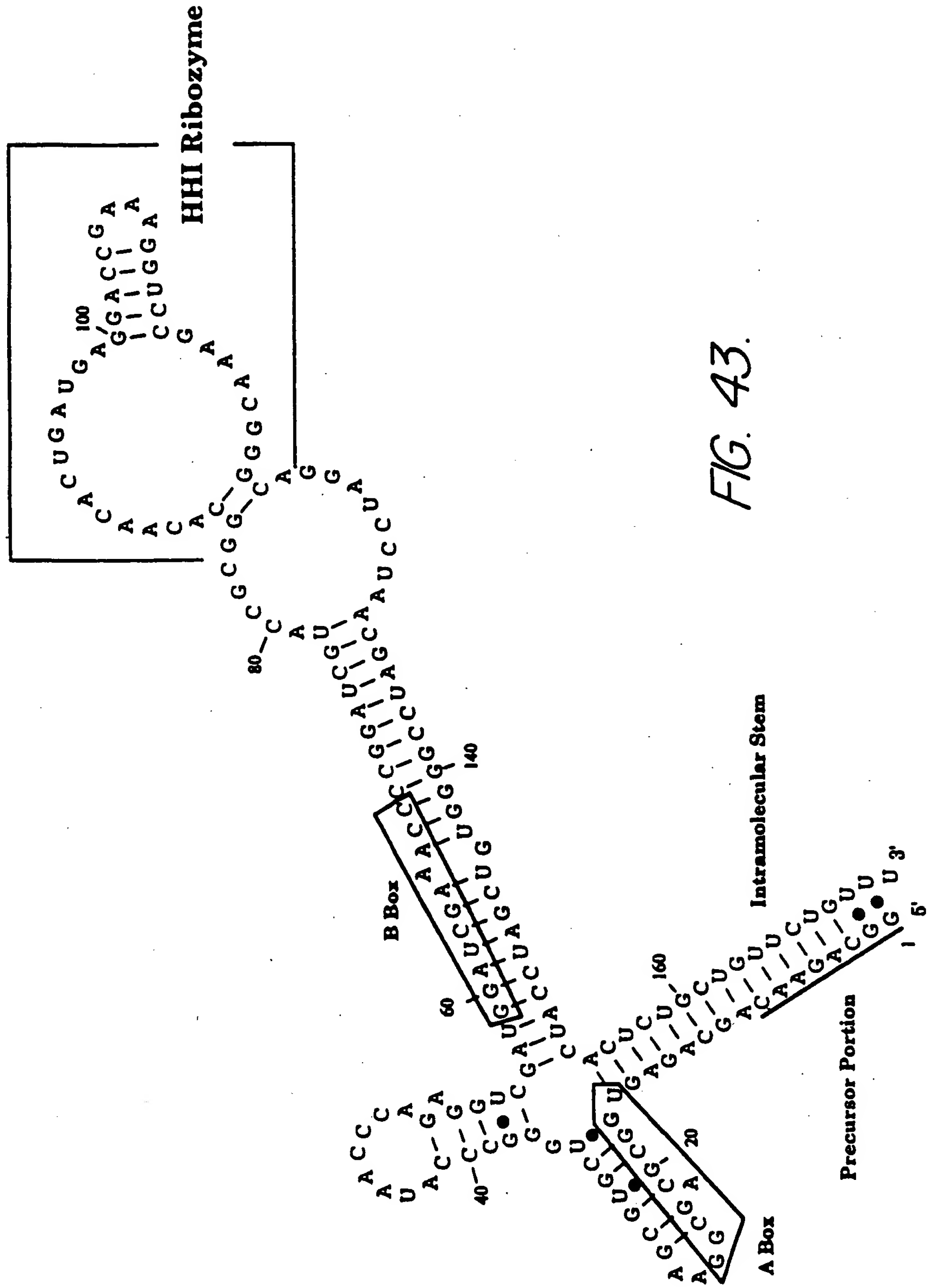

HHI Ribozyme
B Box
A Box
Intramolecular Stem
Precursor Portion

FIG. 43.

FIG. 44. S35 Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50

AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGUGGAU CCACUCUGCU 100

GUUCUGUUU 109

FIG. 45. HHIS35

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50

AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100

GACCGAAAGG UCCGAAACGG GCAGGAUCCA CUCUGCUGUU CUGUUU 146

Underlined bases indicate the HHI ribozyme sequence

FIG. 46. S35 Plus Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50

AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGGGAUC CUAACGAUCC 100

GGGGUGUCGA UCCAUCACUC UGCUGUUCUG UU U 133

FIG. 47. HHIS35 Plus

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50

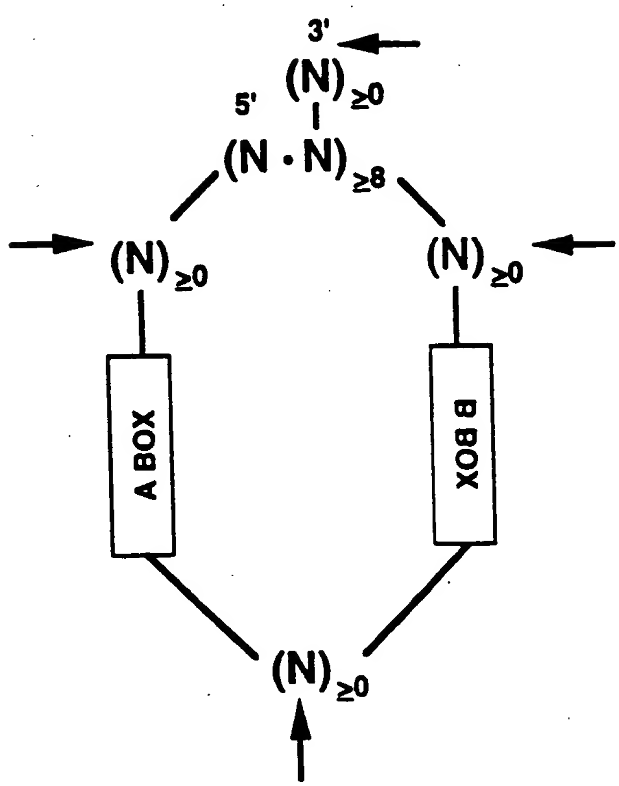
AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100

GACCGAAAGG UCCGAAACGG GCAGGAUCCU AACGAUCCGG GGUGUCGAUC 150

CAUCACUCUG CUGUUCUGUU U 171

Underlined bases indicate the HHI ribozyme sequence

FIG. 48.



A BOX = URGCNNAGYGG
B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) Annu. Review Biochem. 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

→ = Indicates sites at which desired RNAs can be cloned

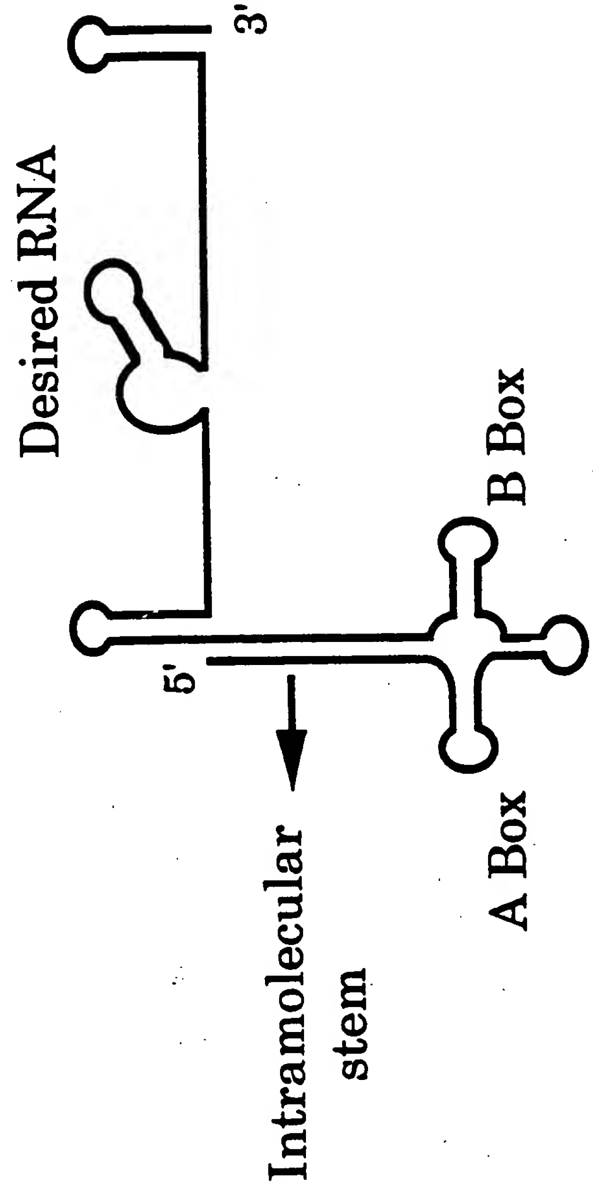


FIG. 49.

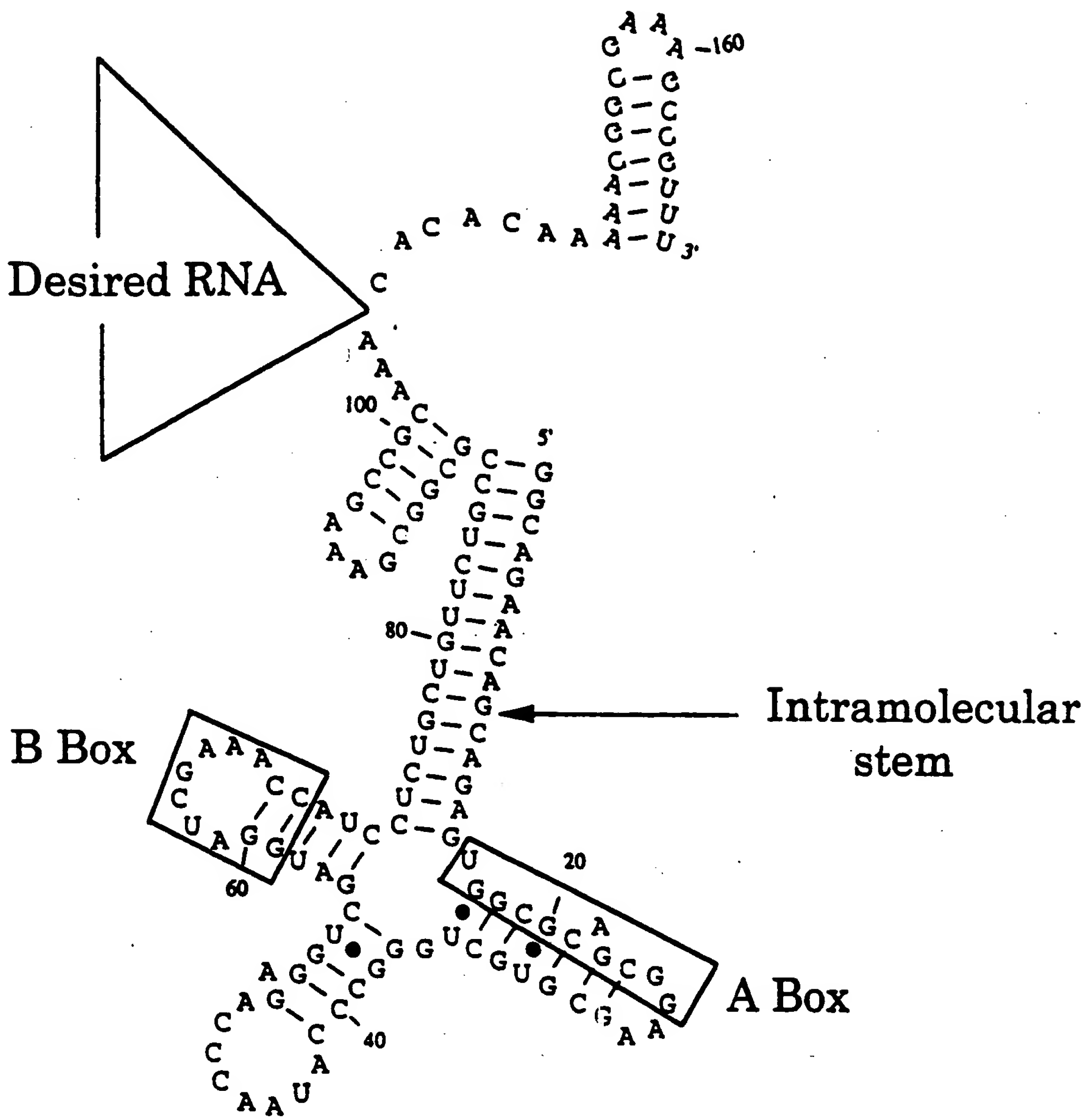

Desired RNA
B Box
Intramolecular stem
A Box
5'
3'
-160
100
80
60
40
20

FIG. 50.

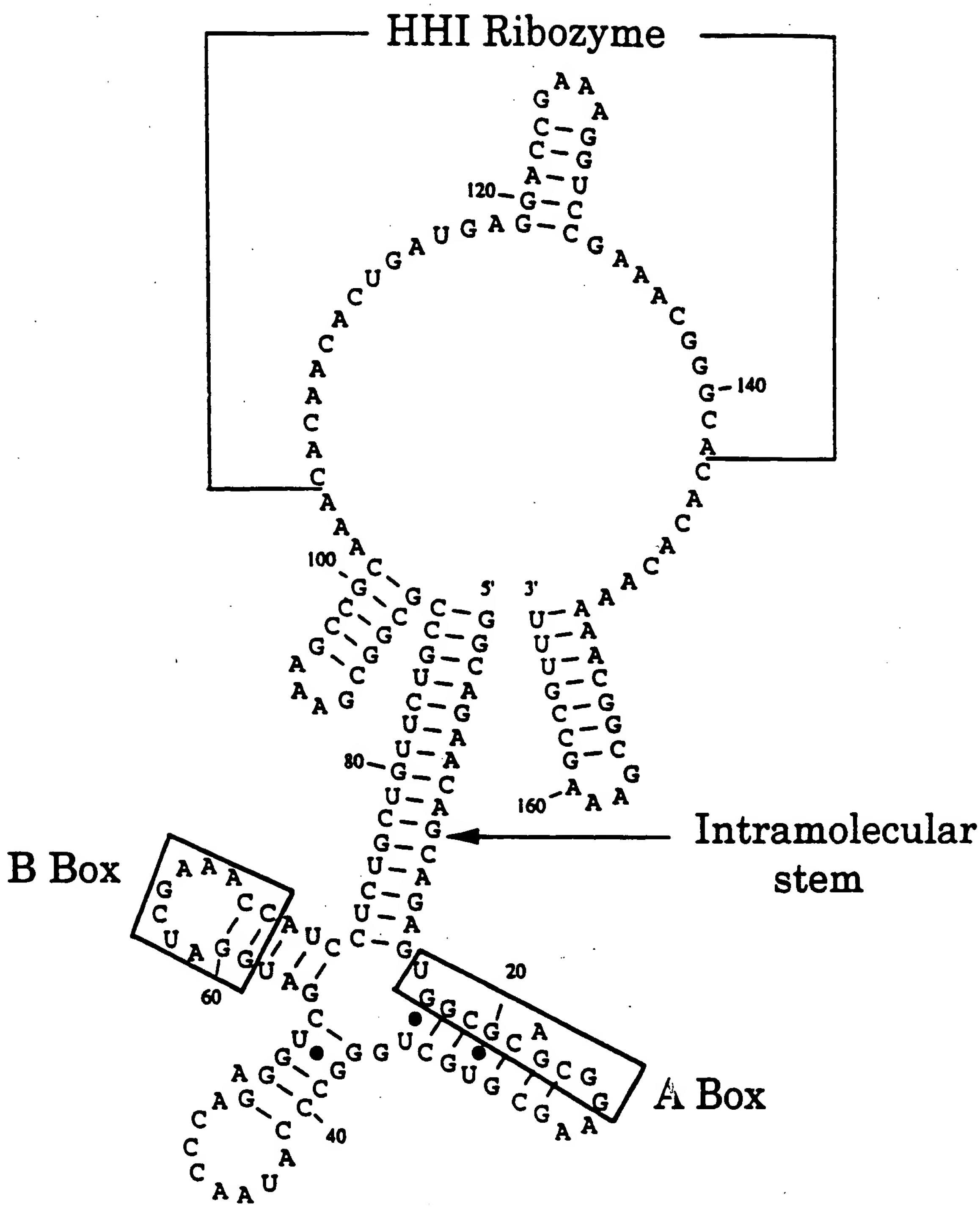

HHI Ribozyme
Intramolecular stem
B Box
A Box
5'
3'
20
40
60
80
100
120
140
160

FIG. 51.

FIG. 52a.

A: TRZ-A

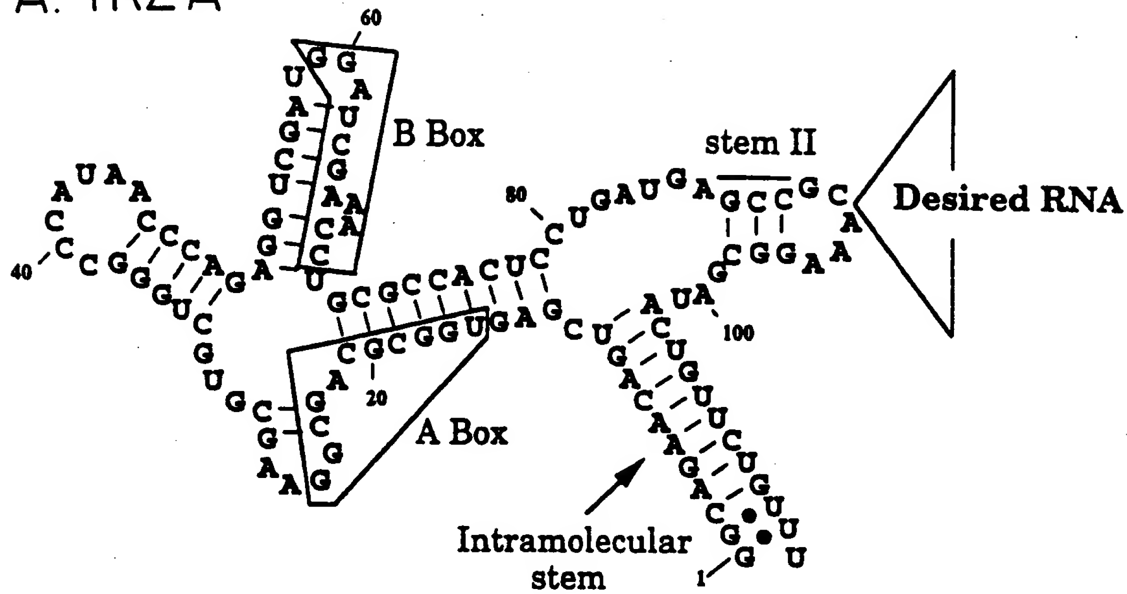
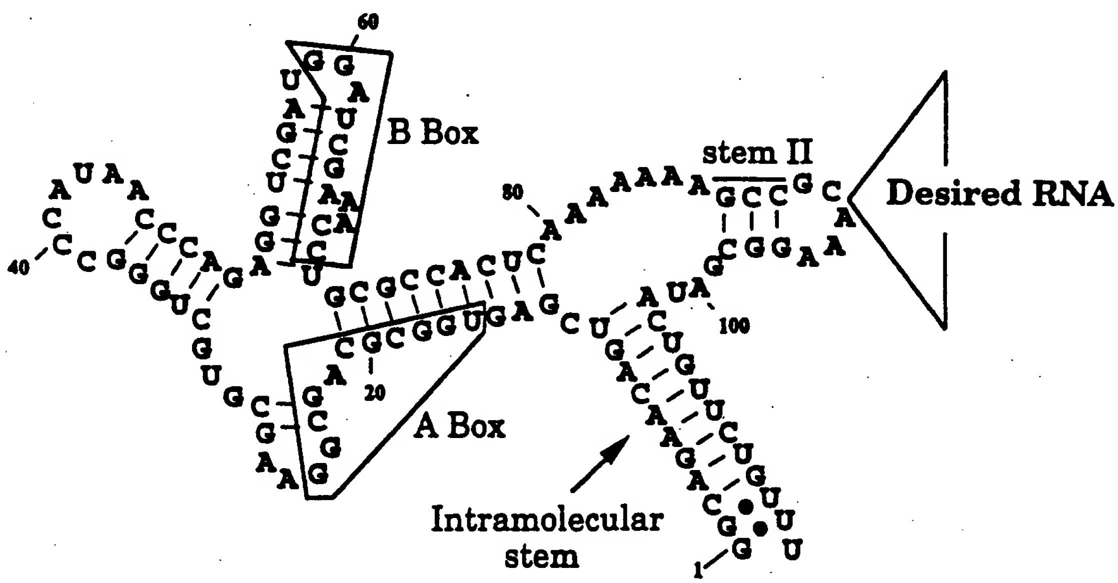


FIG. 52b.

B: TRZ-B



[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

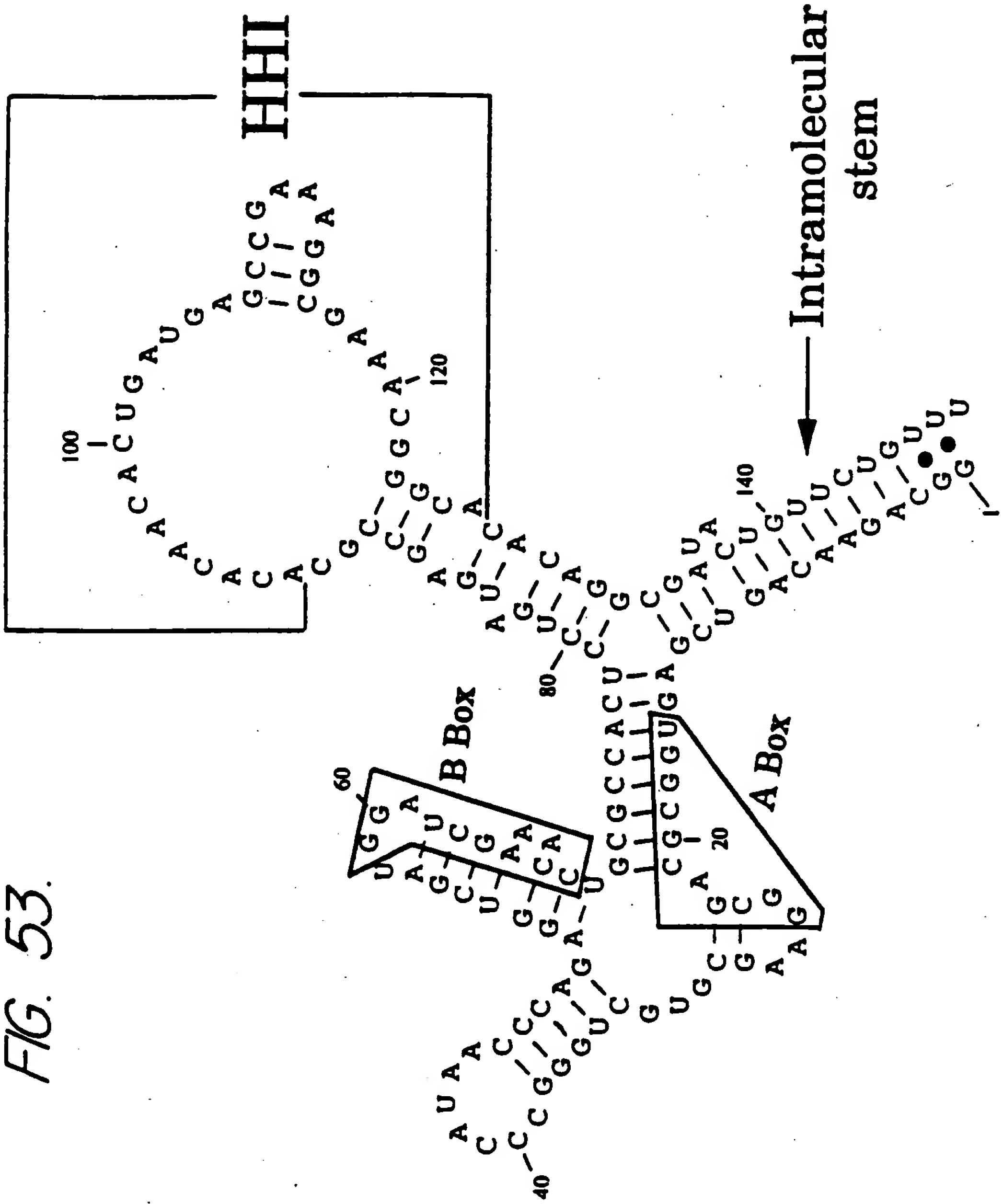


FIG. 53.

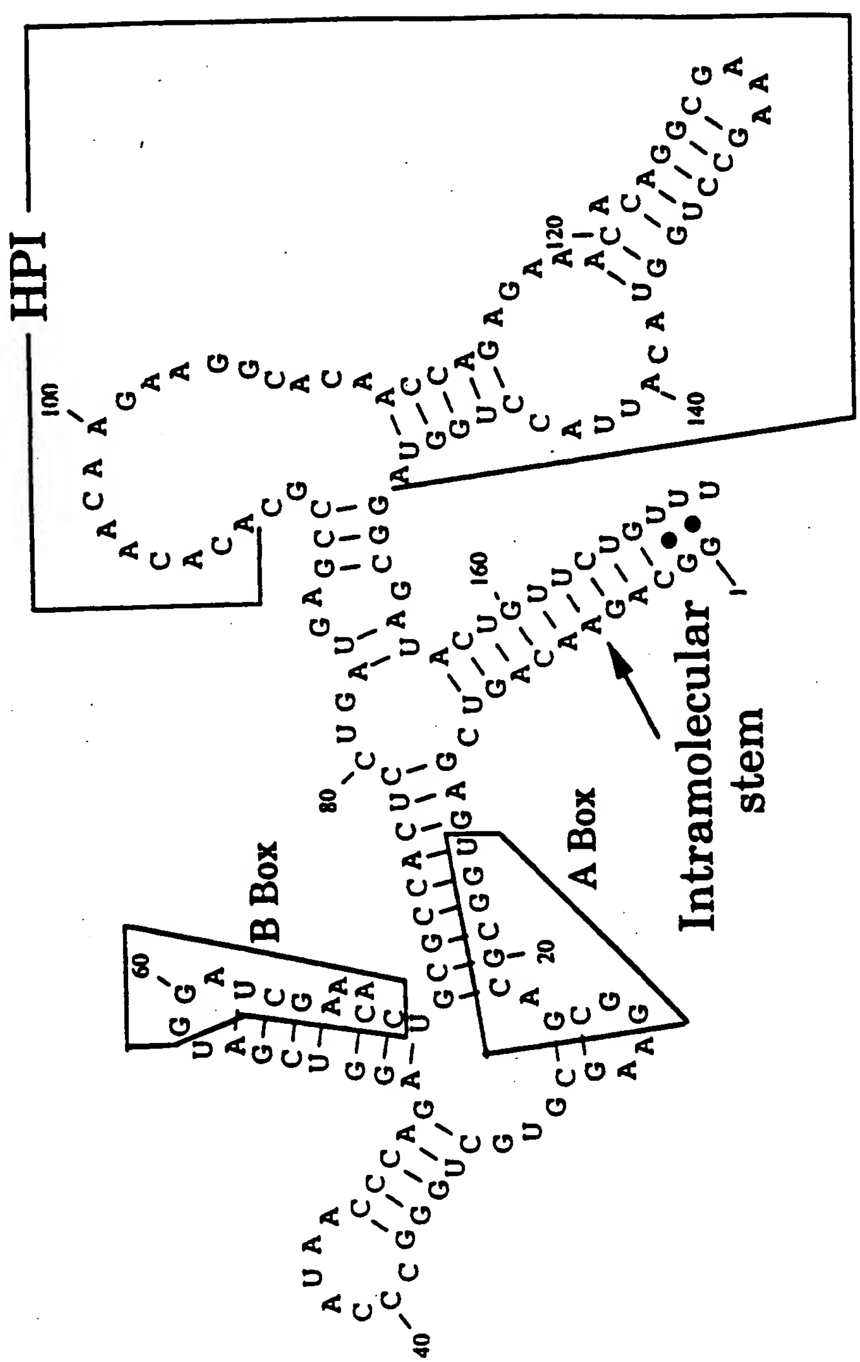
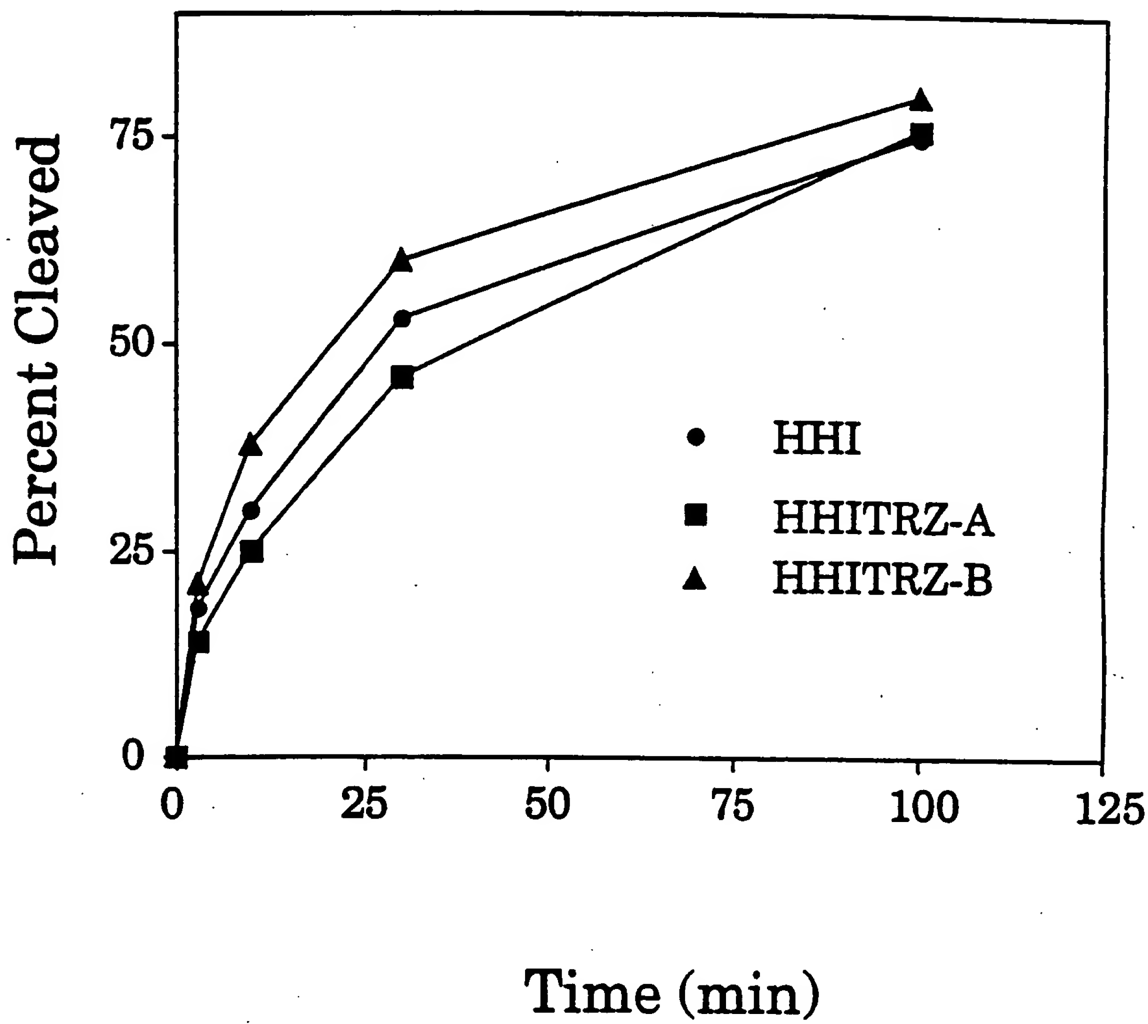


FIG. 54.



Percent Cleaved
0
25
50
75
0
25
50
75
100
125
HHI
HHITRZ-A
HHITRZ-B
Time (min)

FIG. 55.

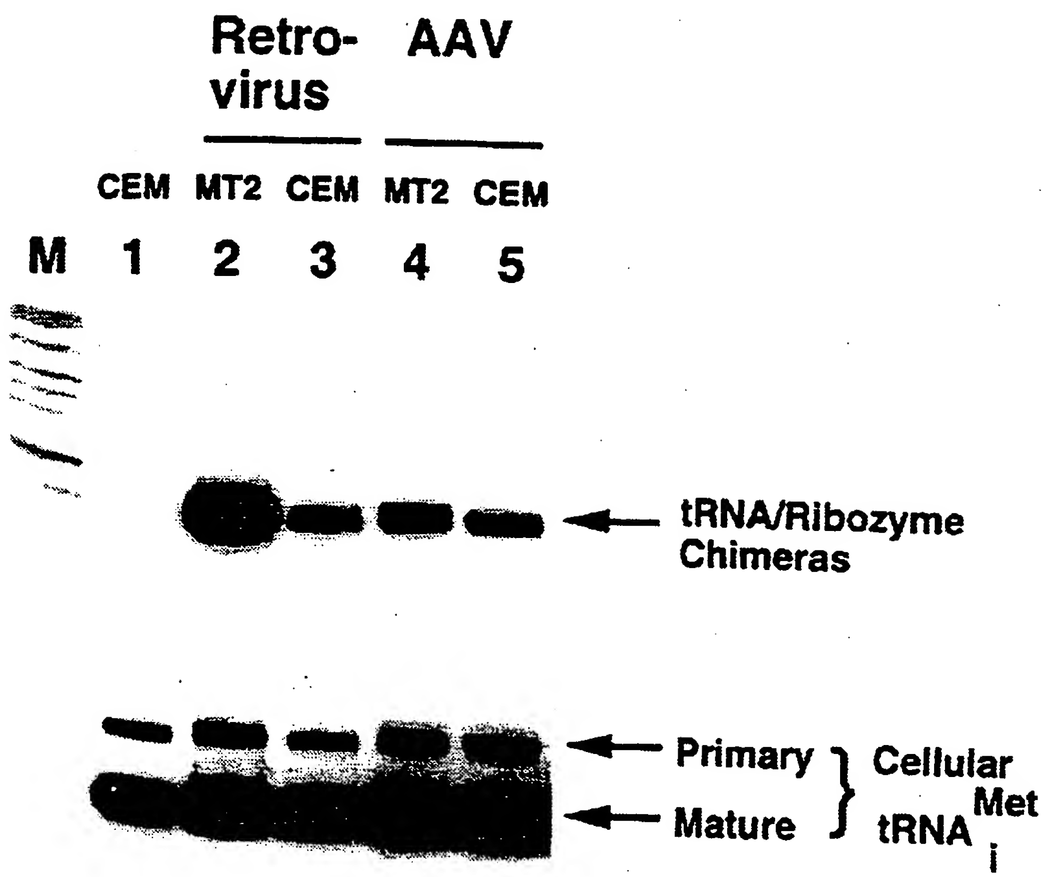

Retro-virus
AAV
CEM
MT2
CEM
MT2
CEM
M
1
2
3
4
5
tRNA/Ribozyme Chimeras
Primary
Mature
Cellular $tRNA_i^{Met}$

FIG. 56.

FIG. 57a.

AAV Vector

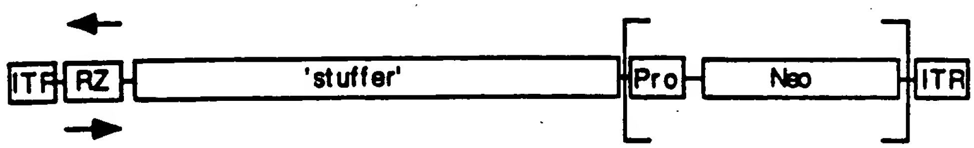
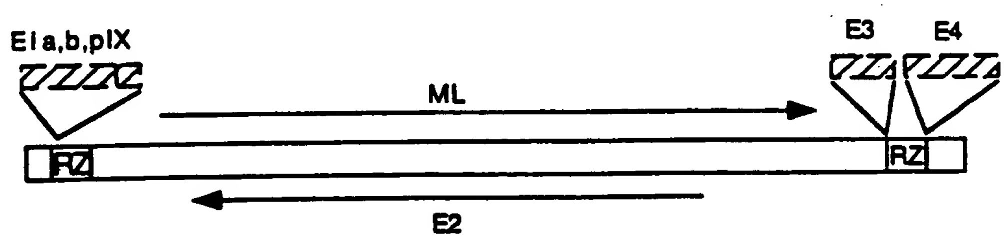


FIG. 57b.

Adenovirus Vector



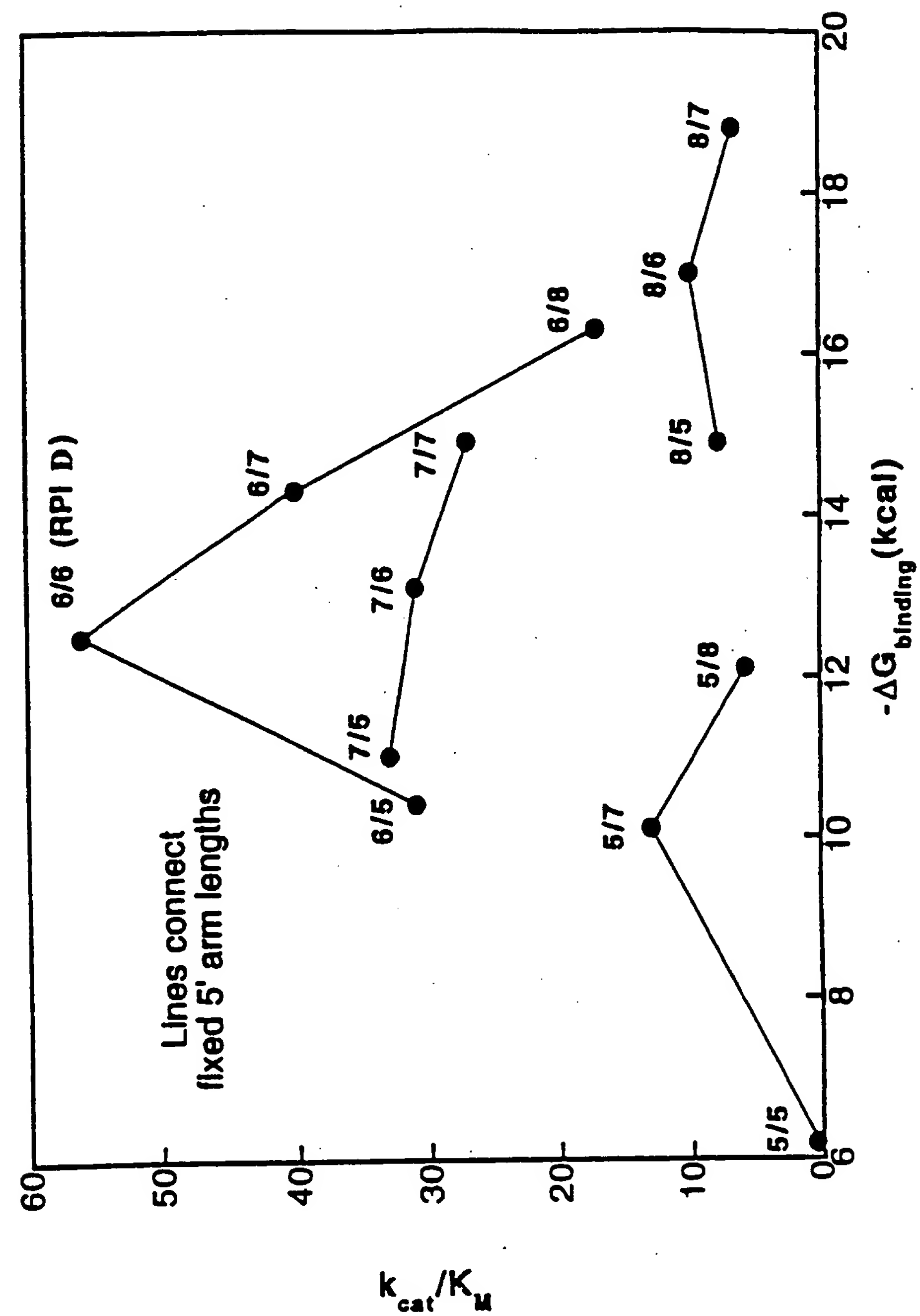

Lines connect
fixed 5' arm lengths
6/6 (RPI D)
6/7
6/8
6/5
7/5
7/6
7/7
8/5
8/6
8/7
5/5
5/7
5/8
k_{cat}/K_M
$-\Delta G_{binding}$(kcal)
0
10
20
30
40
50
60
6
8
10
12
14
16
18
20

FIG. 58.

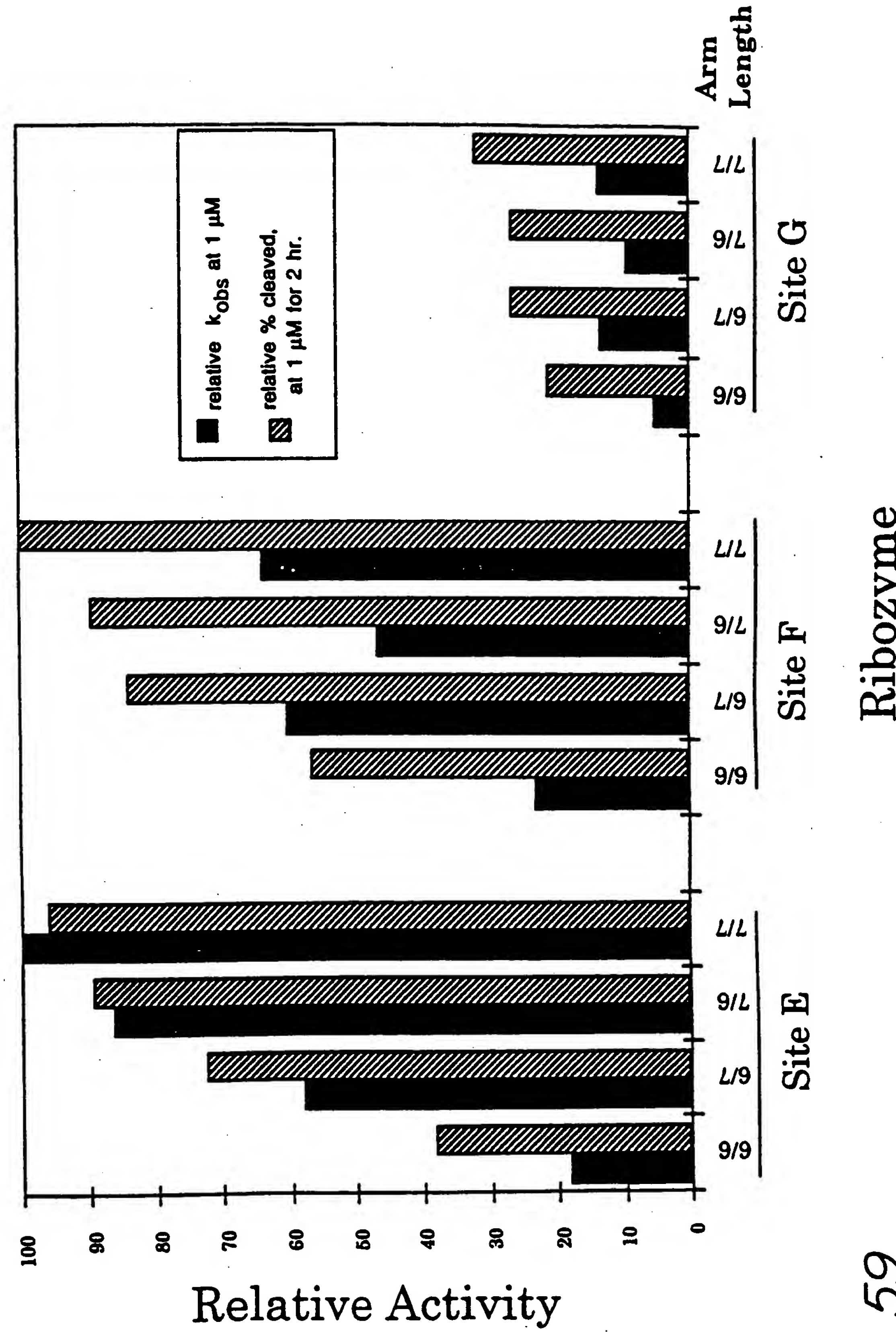

relative k_{obs} at 1 µM
relative % cleaved, at 1 µM for 2 hr.
Relative Activity
0
10
20
30
40
50
60
70
80
90
100
Arm Length
9/9
7/9
9/7
7/7
Site E
Site F
Site G
Ribozyme

FIG. 59.

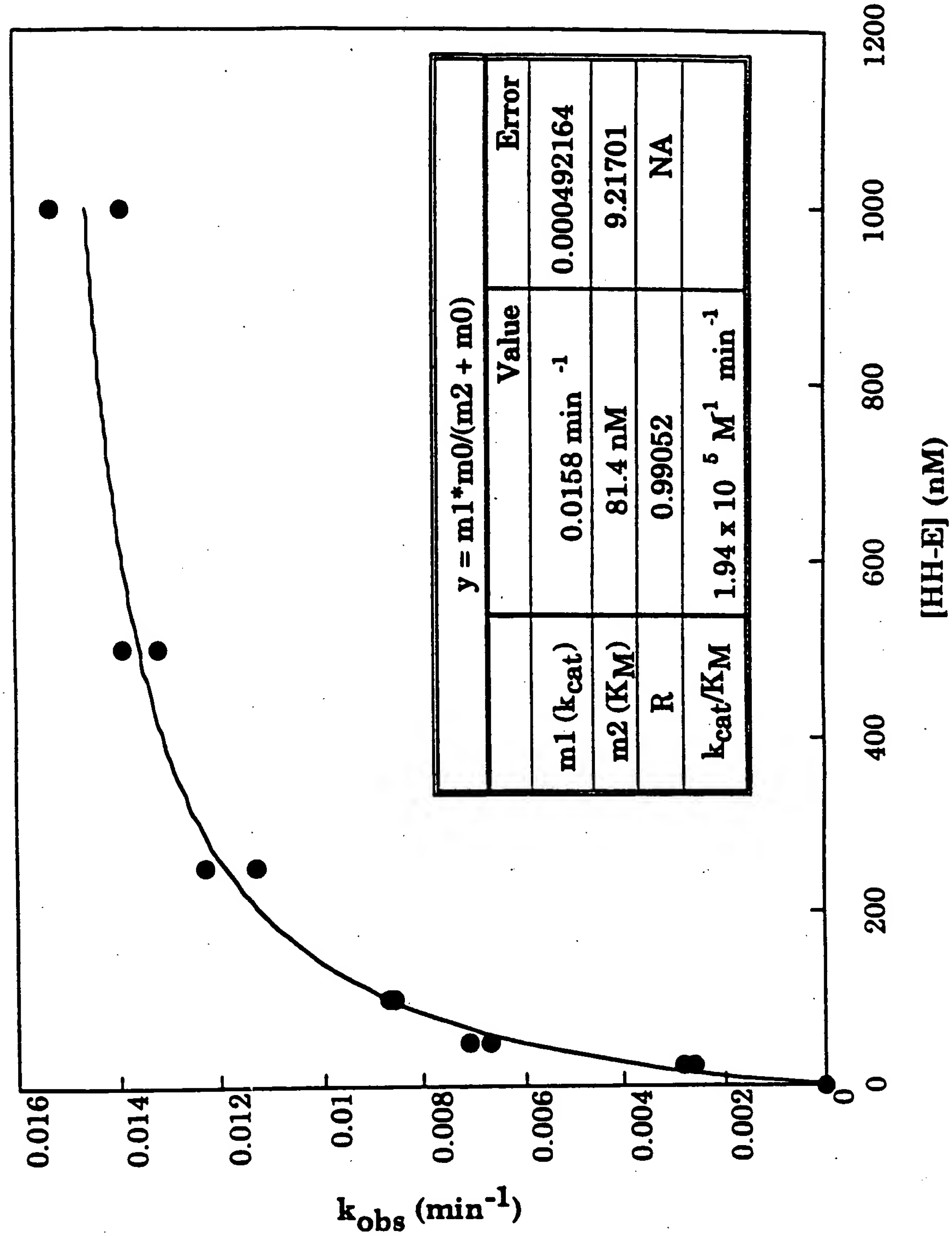

y = m1*m0/(m2 + m0)
Value
Error
m1 (k_{cat})
0.0158 min^{-1}
0.000492164
m2 (K_M)
81.4 nM
9.21701
R
0.99052
NA
k_{cat}/K_M
$1.94 \times 10^5\ M^{-1}\ min^{-1}$
k_{obs} (min^{-1})
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
200
400
600
800
1000
1200
[HH-E] (nM)

FIG. 60.

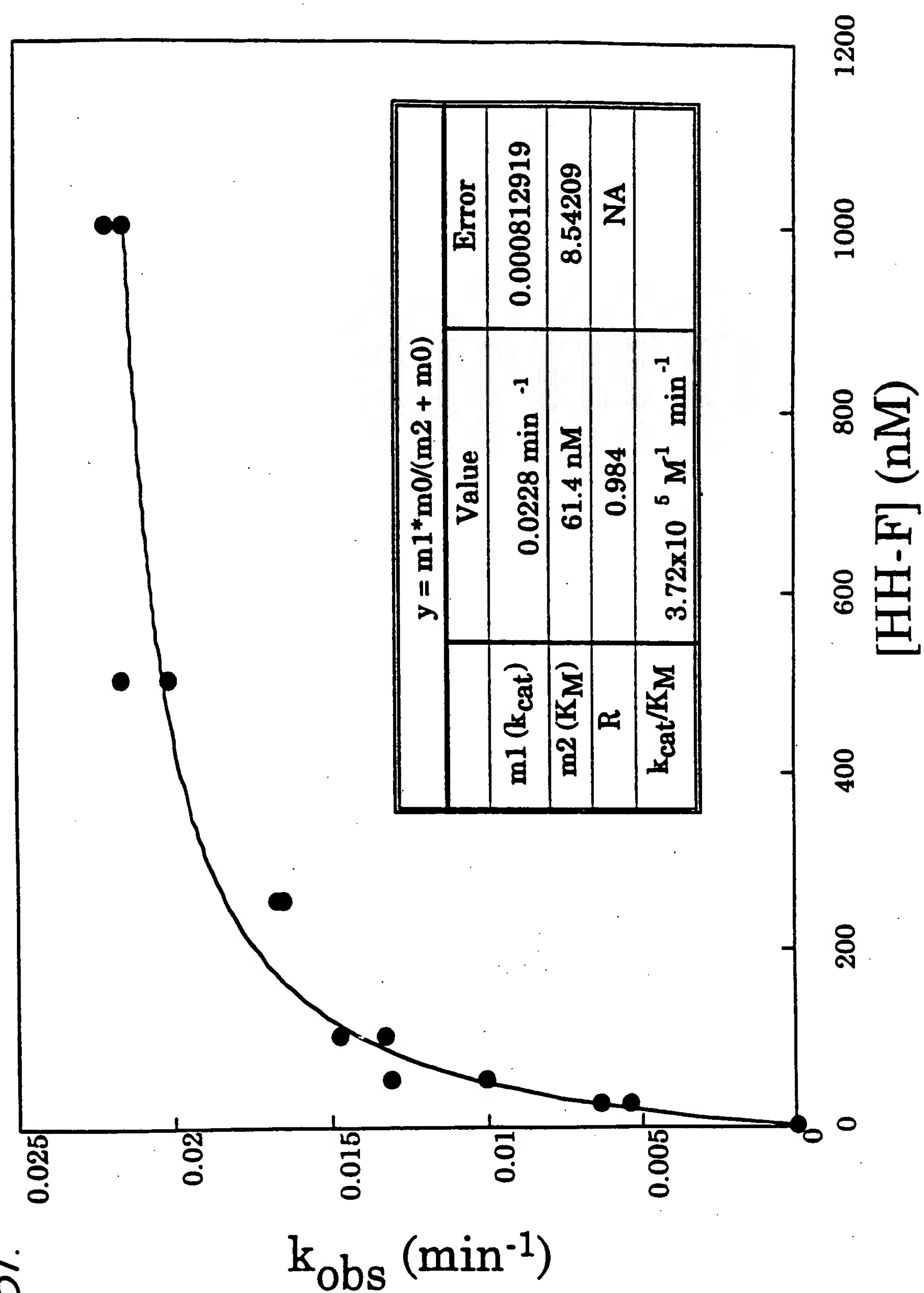

y = m1*m0/(m2 + m0)
Value
Error
m1 (kcat)
0.0228 min -1
0.000812919
m2 (KM)
61.4 nM
8.54209
R
0.984
NA
kcat/KM
3.72x10 5 M-1 min-1
kobs (min-1)
0.025
0.02
0.015
0.01
0.005
0
0
200
400
600
800
1000
1200
[HH-F] (nM)

FIG. 61.

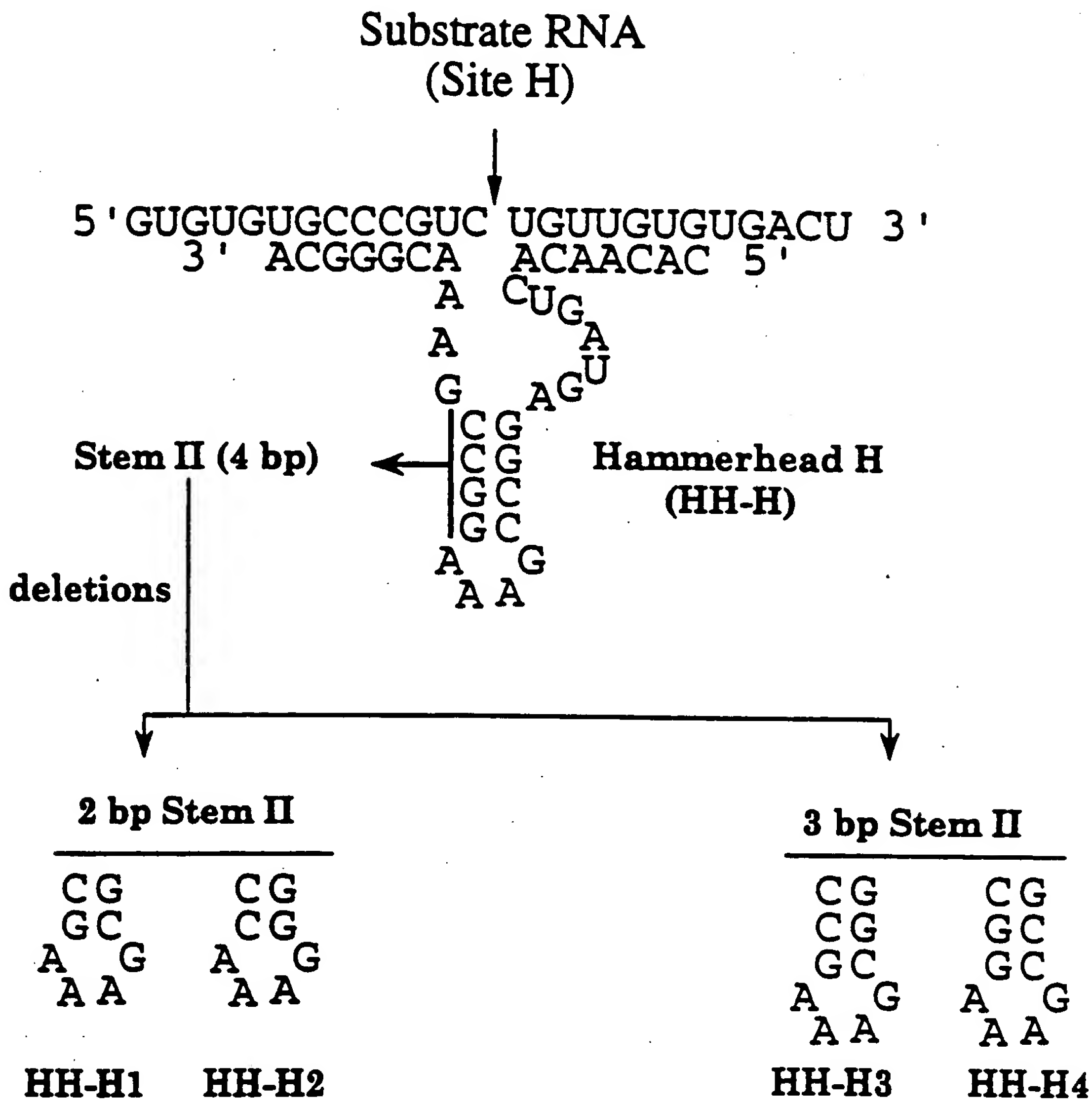
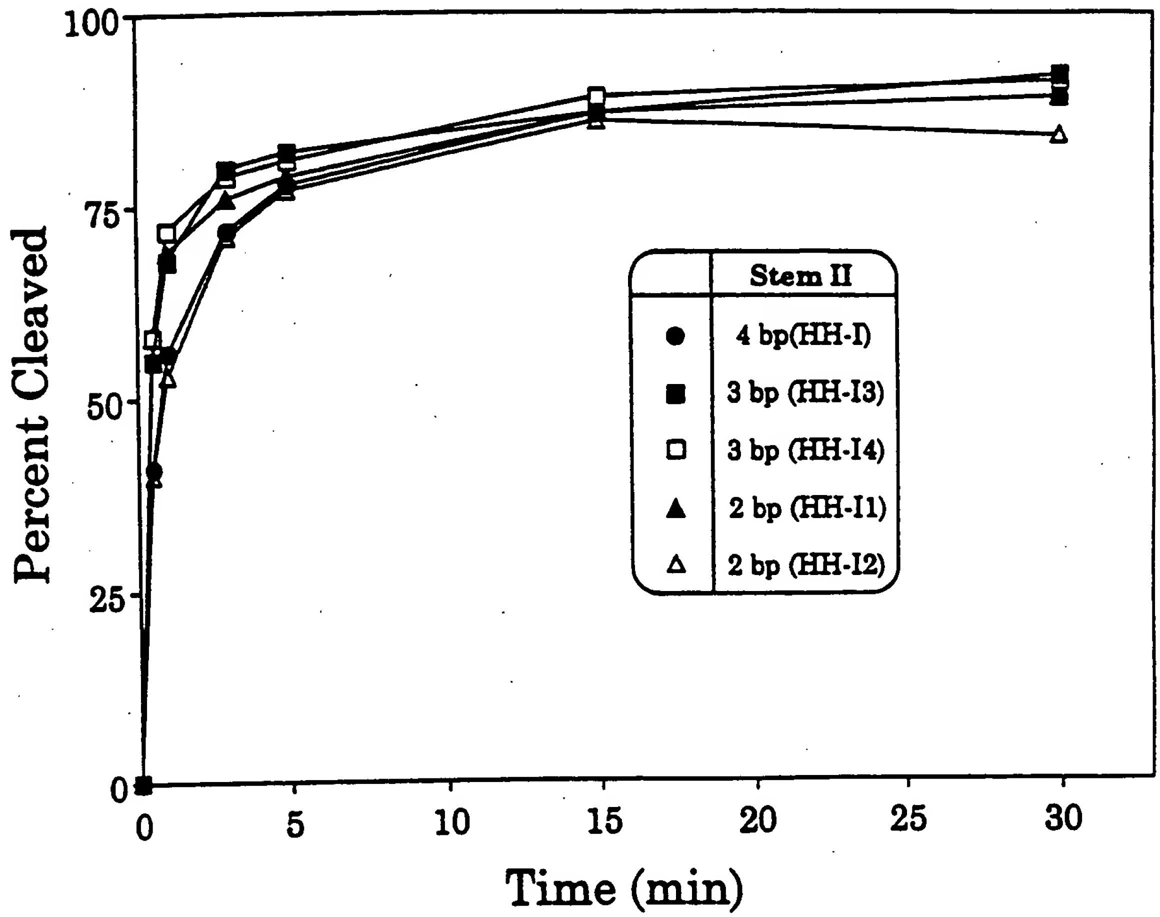

Substrate RNA
(Site H)
5'GUGUGUGCCCGUC UGUUGUGUGACU 3'
3' ACGGGCA ACAACAC 5'
Stem II (4 bp)
Hammerhead H
(HH-H)
deletions
2 bp Stem II
3 bp Stem II
HH-H1
HH-H2
HH-H3
HH-H4

FIG. 62.



[Ribozyme] = 25 nM [Substrate] = ~ 1 nM

FIG. 63.

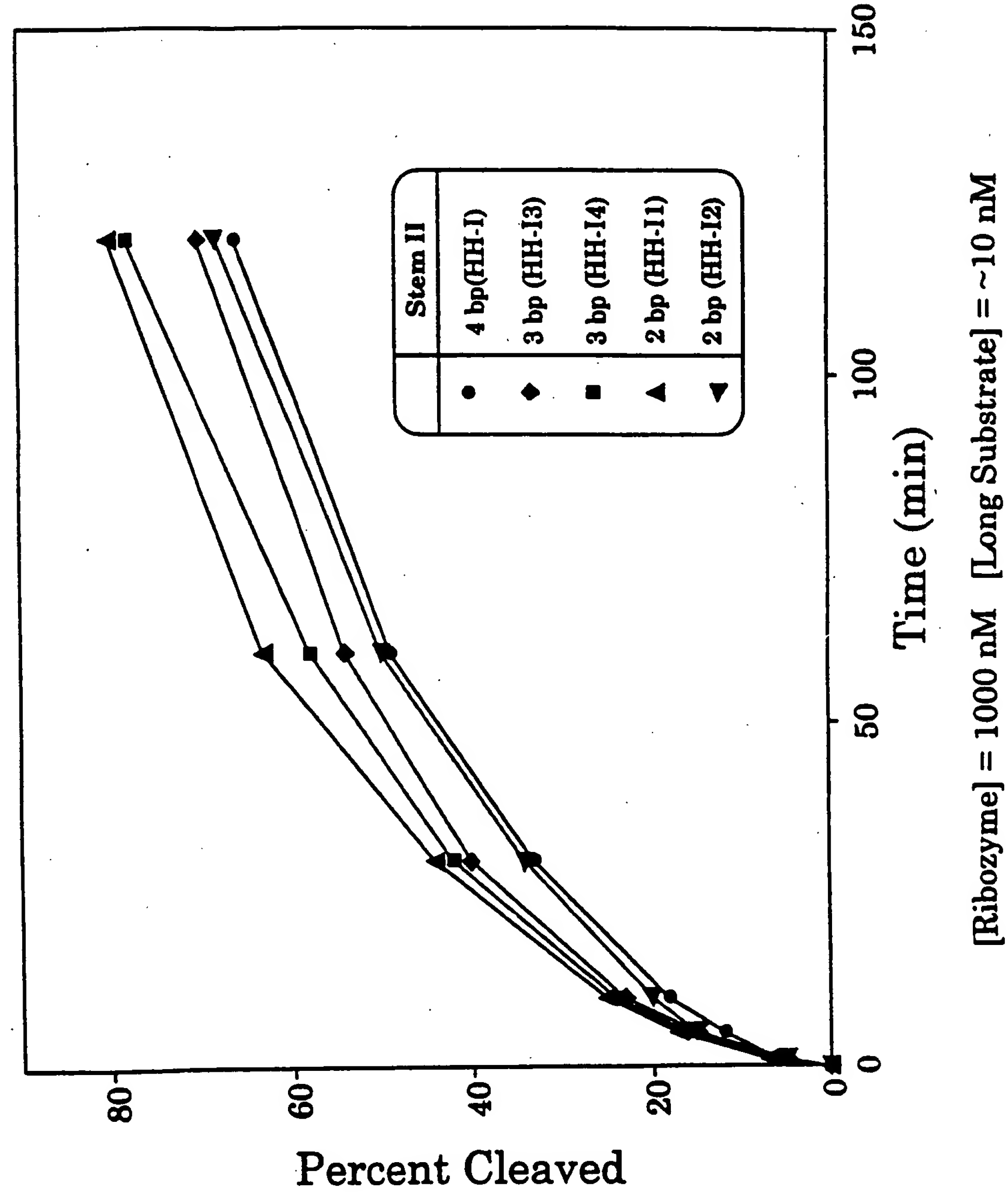

Stem II
4 bp(HH-I)
3 bp (HH-I3)
3 bp (HH-I4)
2 bp (HH-I1)
2 bp (HH-I2)
Percent Cleaved
0
20
40
60
80
Time (min)
0
50
100
150
[Ribozyme] = 1000 nM [Long Substrate] = ~10 nM

FIG. 64.

FIG. 65a.

Substrate RNA (site J)

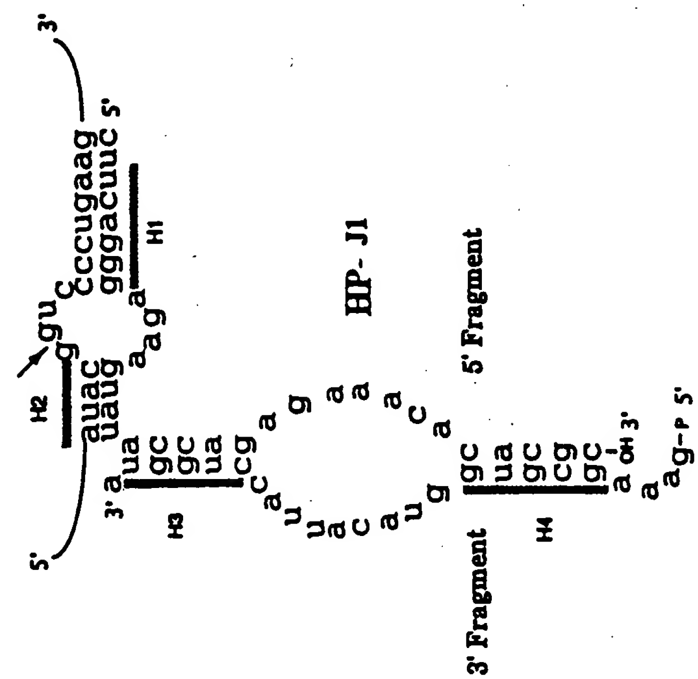
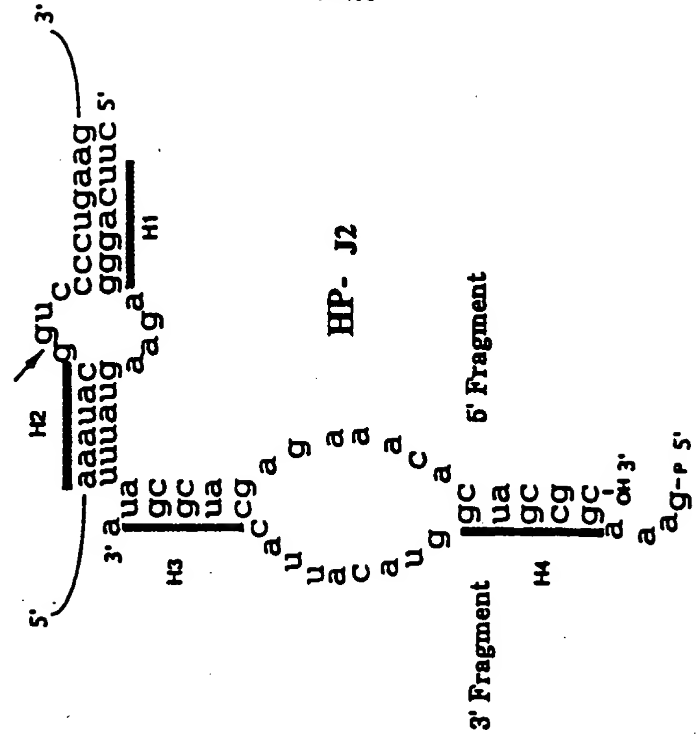


FIG. 65b.

Substrate RNA (site J)



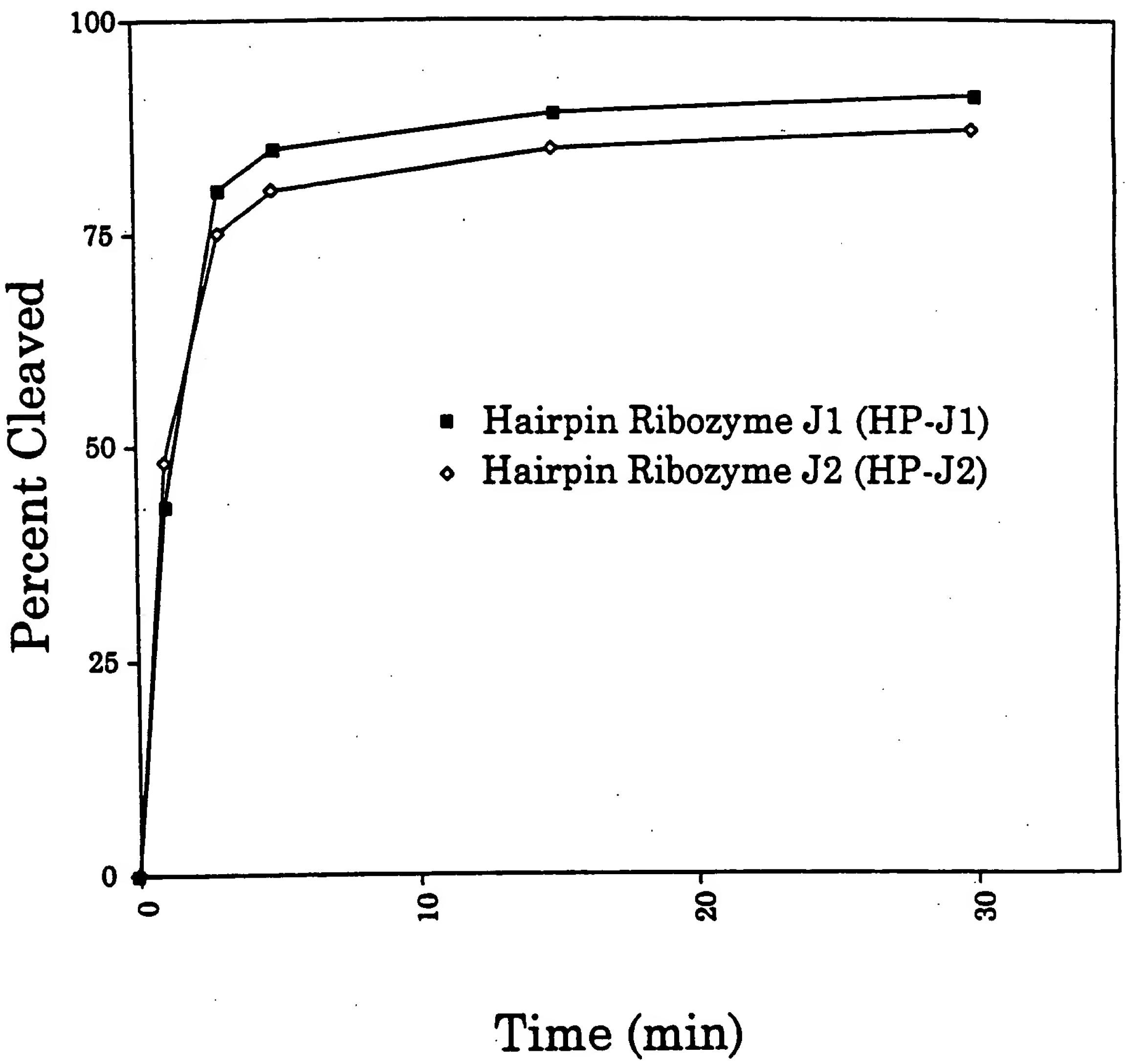

100
75
50
25
0
Percent Cleaved
0
10
20
30
Time (min)
Hairpin Ribozyme J1 (HP-J1)
Hairpin Ribozyme J2 (HP-J2)

FIG. 66.

FIG. 67b.

Substrate RNA

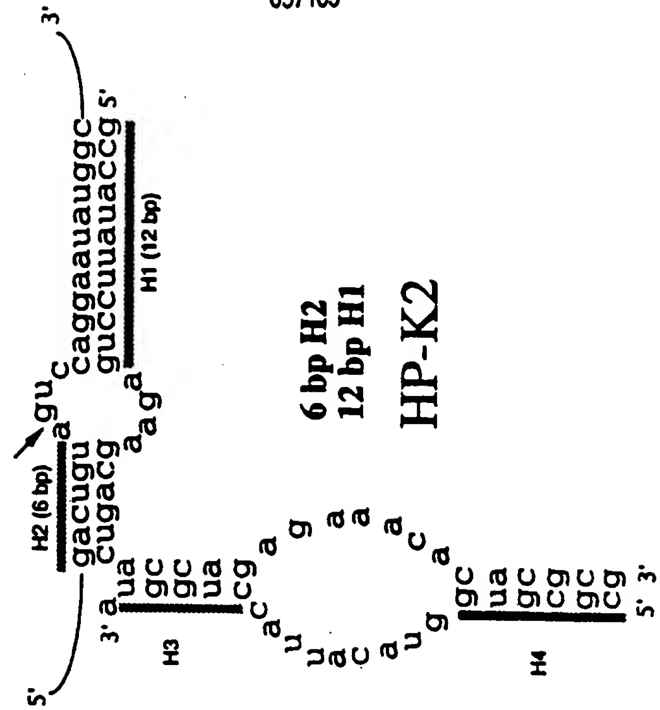
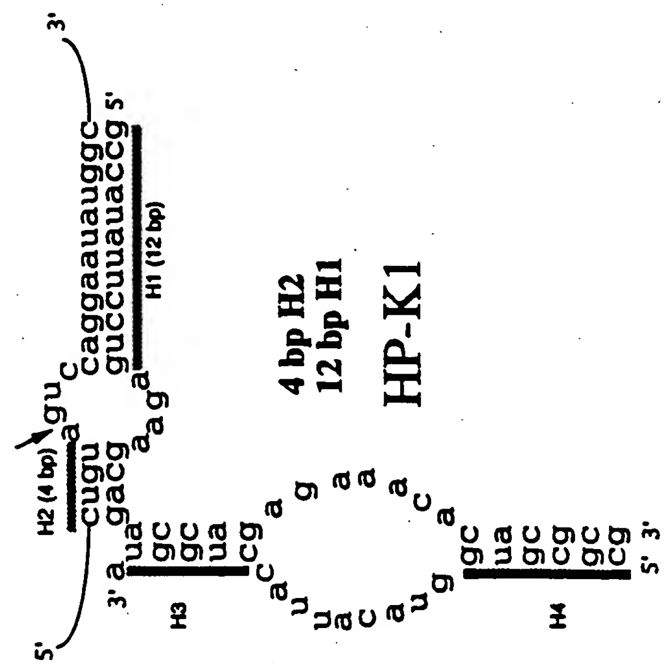


FIG. 67a.

Substrate RNA



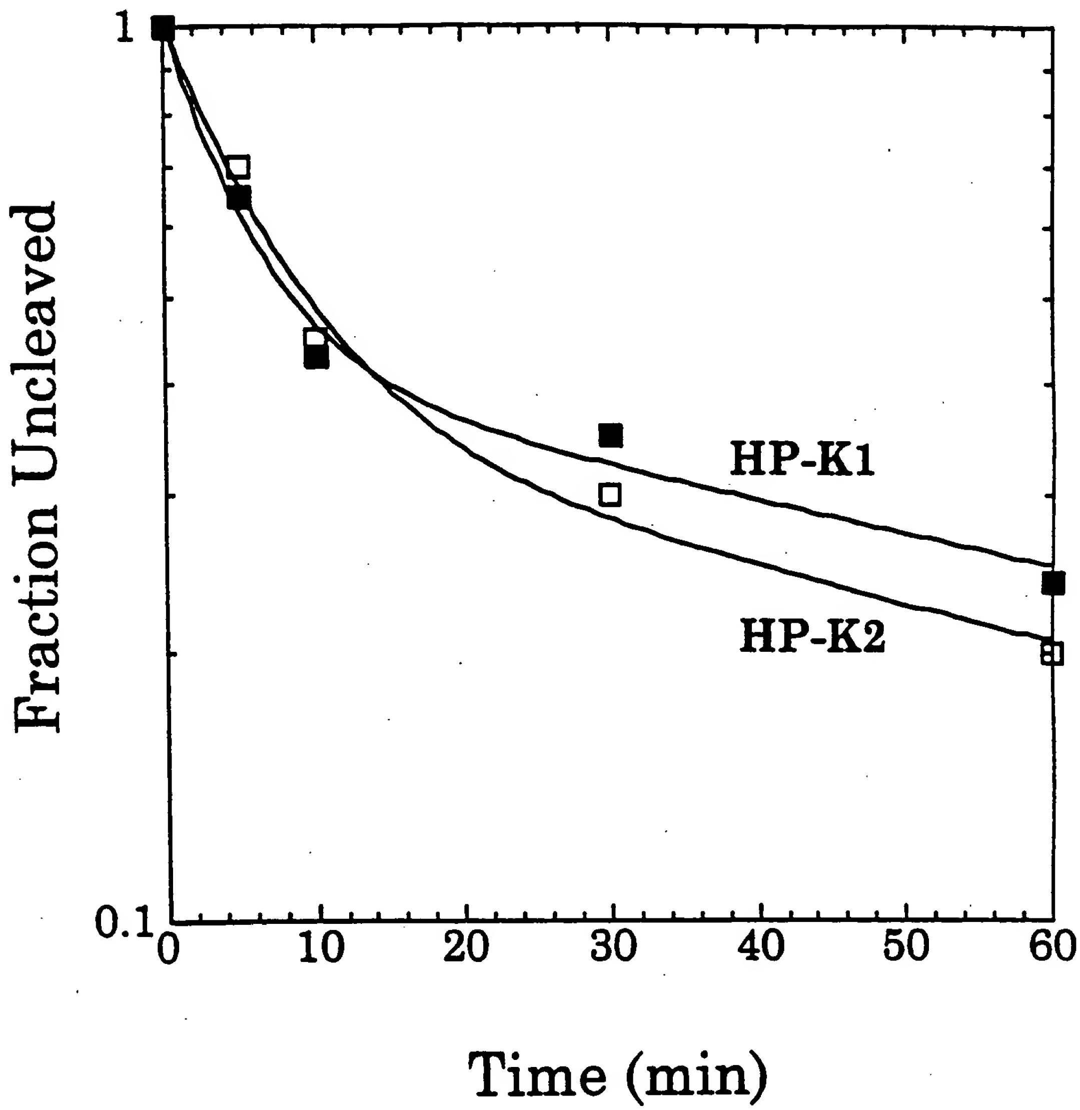


FIG. 68.

FIG. 69a.

Substrate RNA

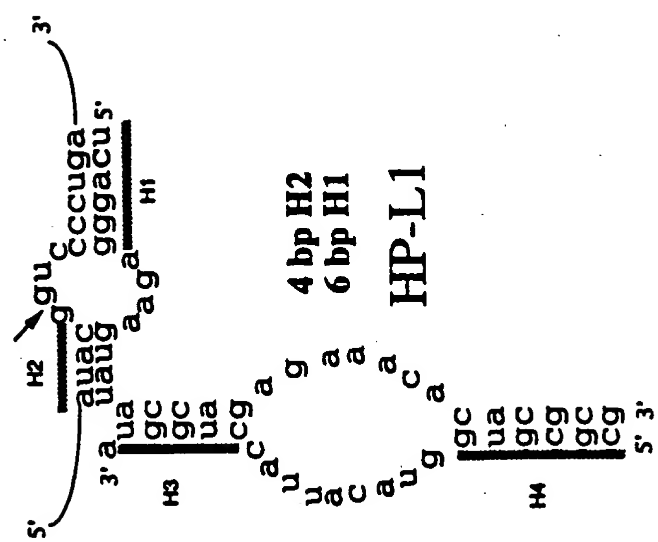
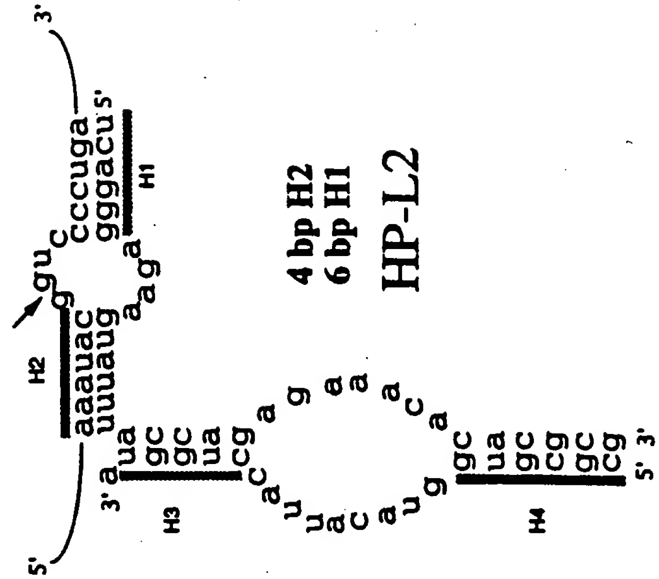


FIG. 69b.

Substrate RNA



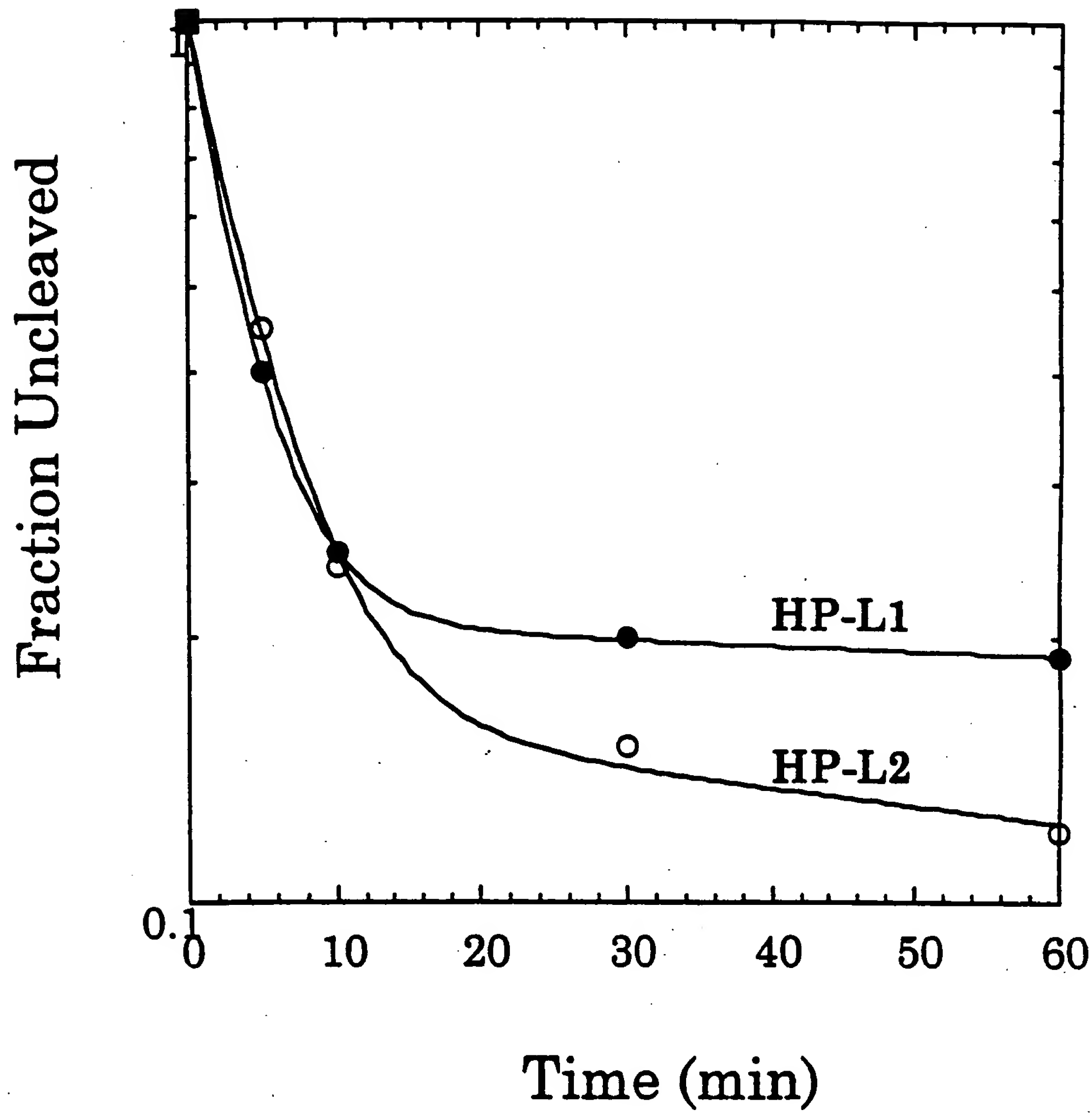


FIG. 70.

FIG. 7b.

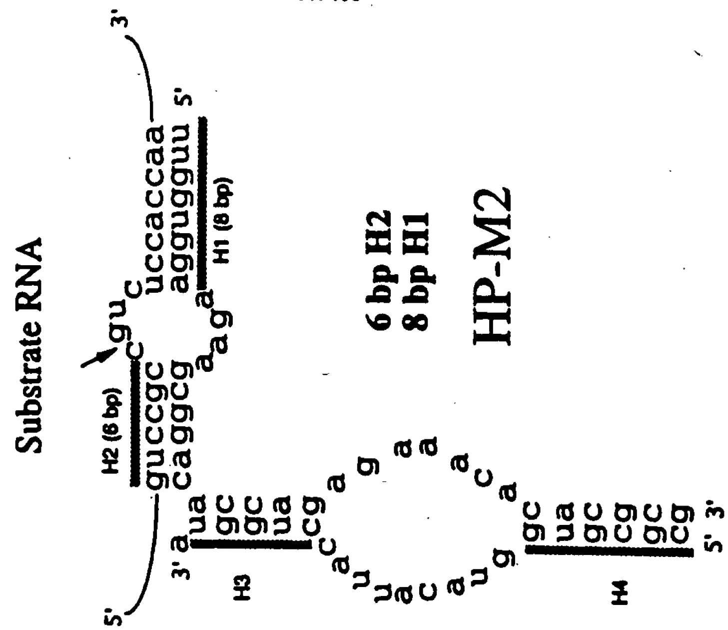
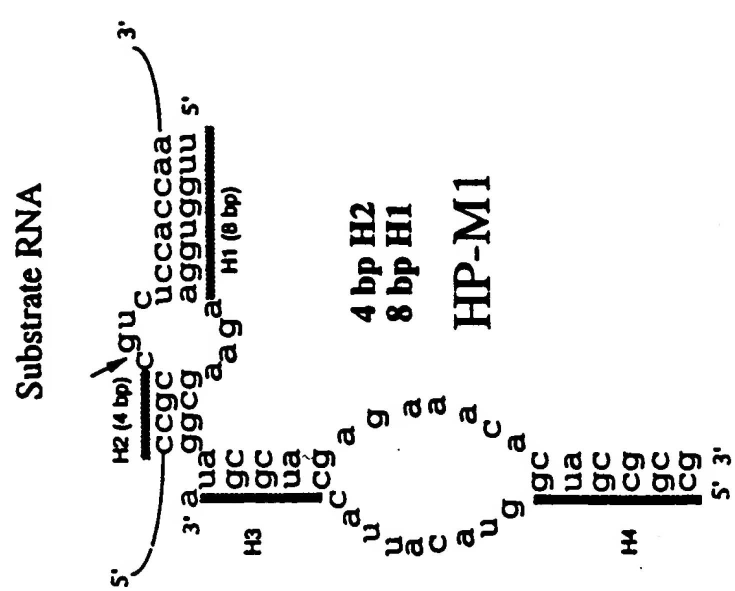

Substrate RNA
H2 (6 bp)
H1 (8 bp)
H3
H4
6 bp H2
8 bp H1
HP-M2

FIG. 7a.


Substrate RNA
H2 (4 bp)
H1 (8 bp)
H3
H4
4 bp H2
8 bp H1
HP-M1

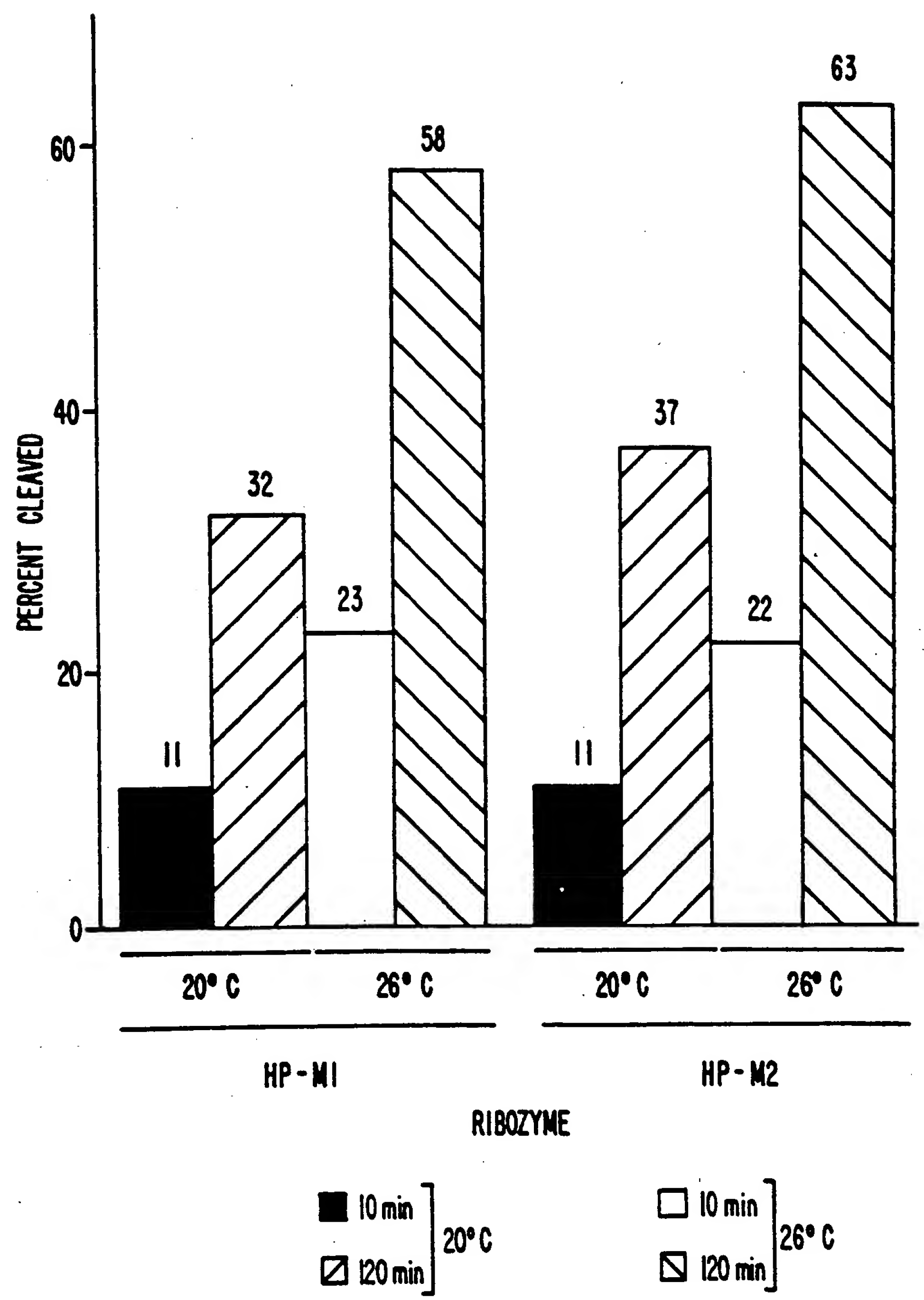

PERCENT CLEAVED
0
20
40
60
11
32
23
58
11
37
22
63
20° C
26° C
20° C
26° C
HP-M1
HP-M2
RIBOZYME
10 min
120 min
20° C
10 min
120 min
26° C

FIG. 72.

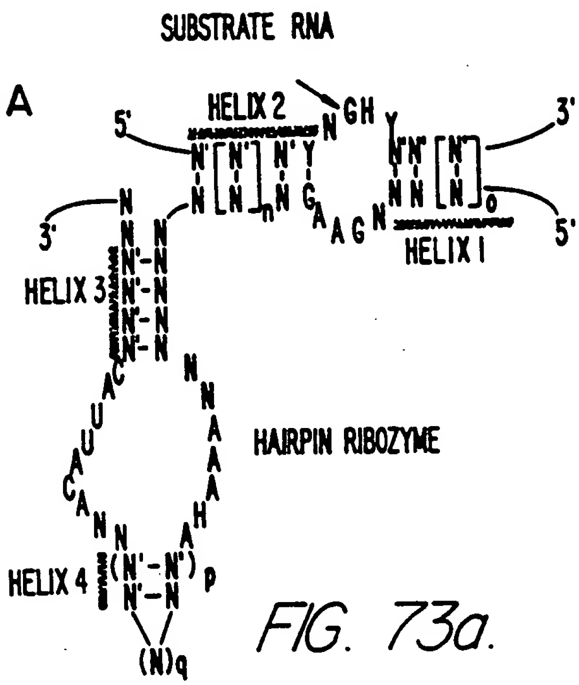


FIG. 73a.

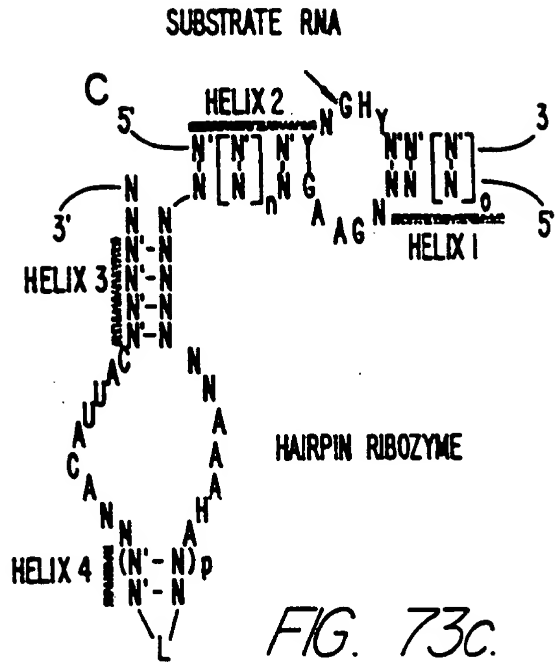


FIG. 73c.

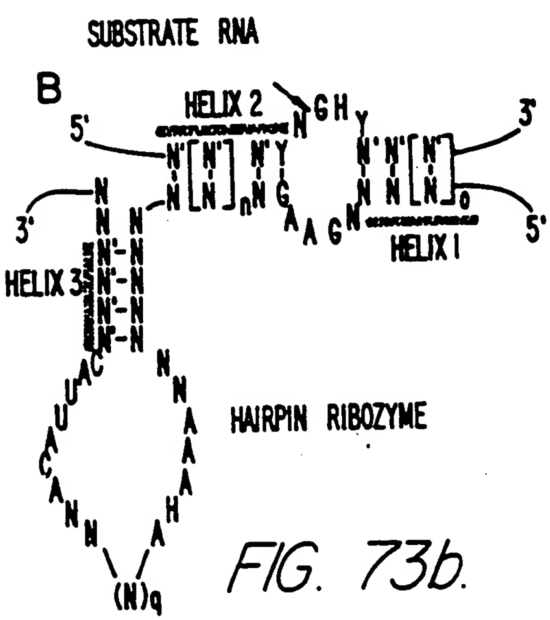


FIG. 73b.

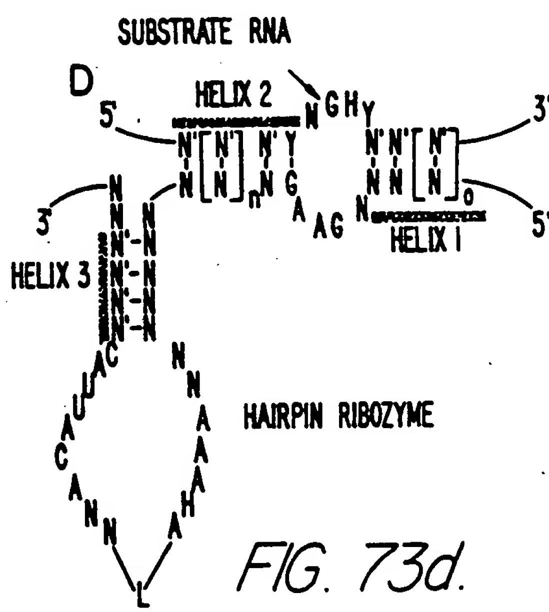
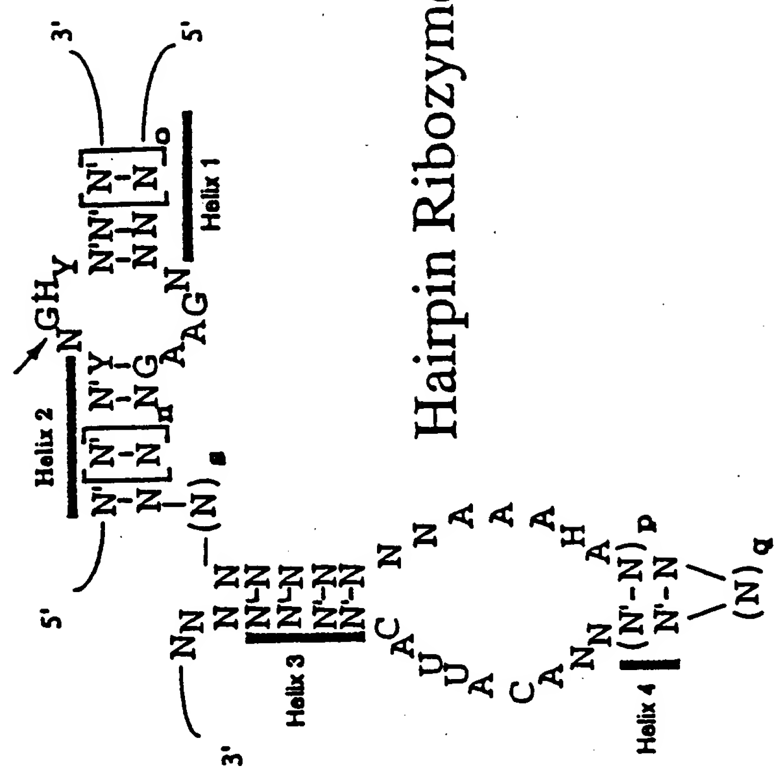


FIG. 73d.

FIG. 74b.

B

Substrate RNA

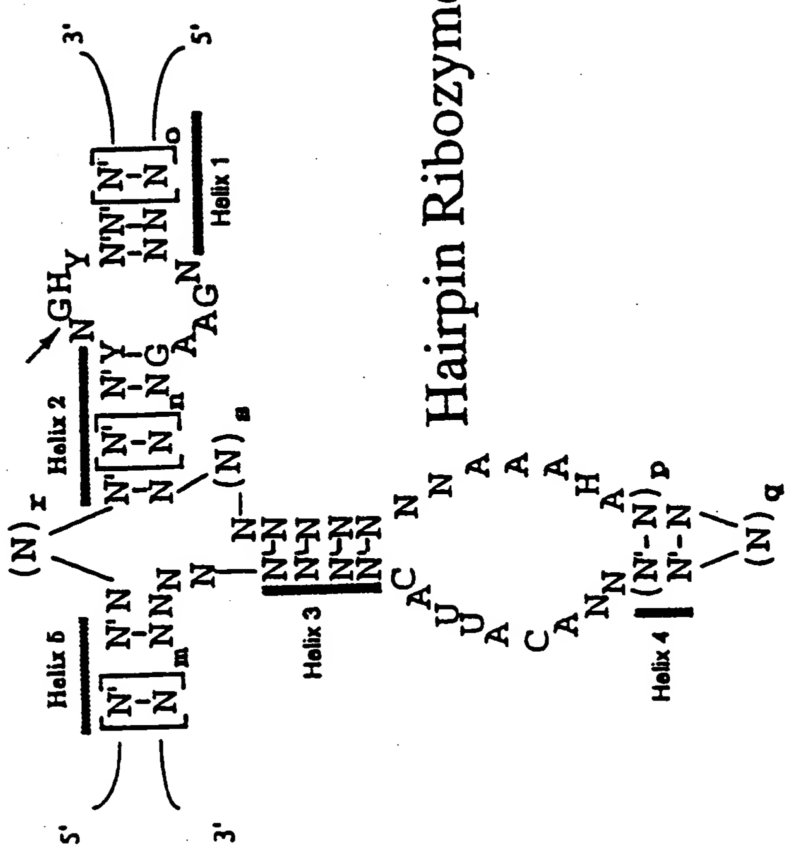


Hairpin Ribozyme

FIG. 74a.

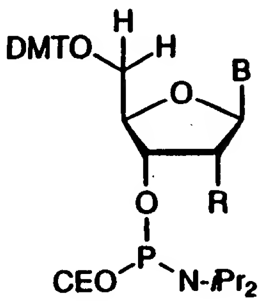
A

Substrate RNA



Hairpin Ribozyme

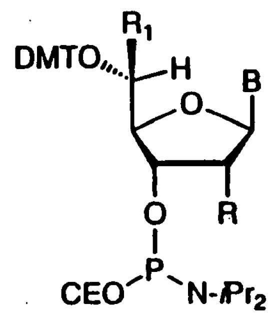
FIG. 75a.



1

D-Ribose Family

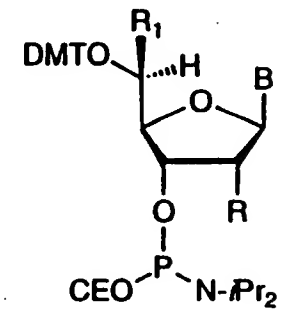
FIG. 75b.



2

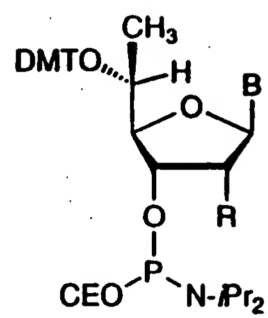
D-Allose Family

FIG. 75c.



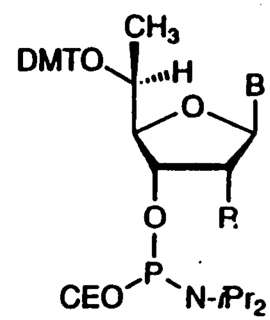
3

L-Talose Family



29-32

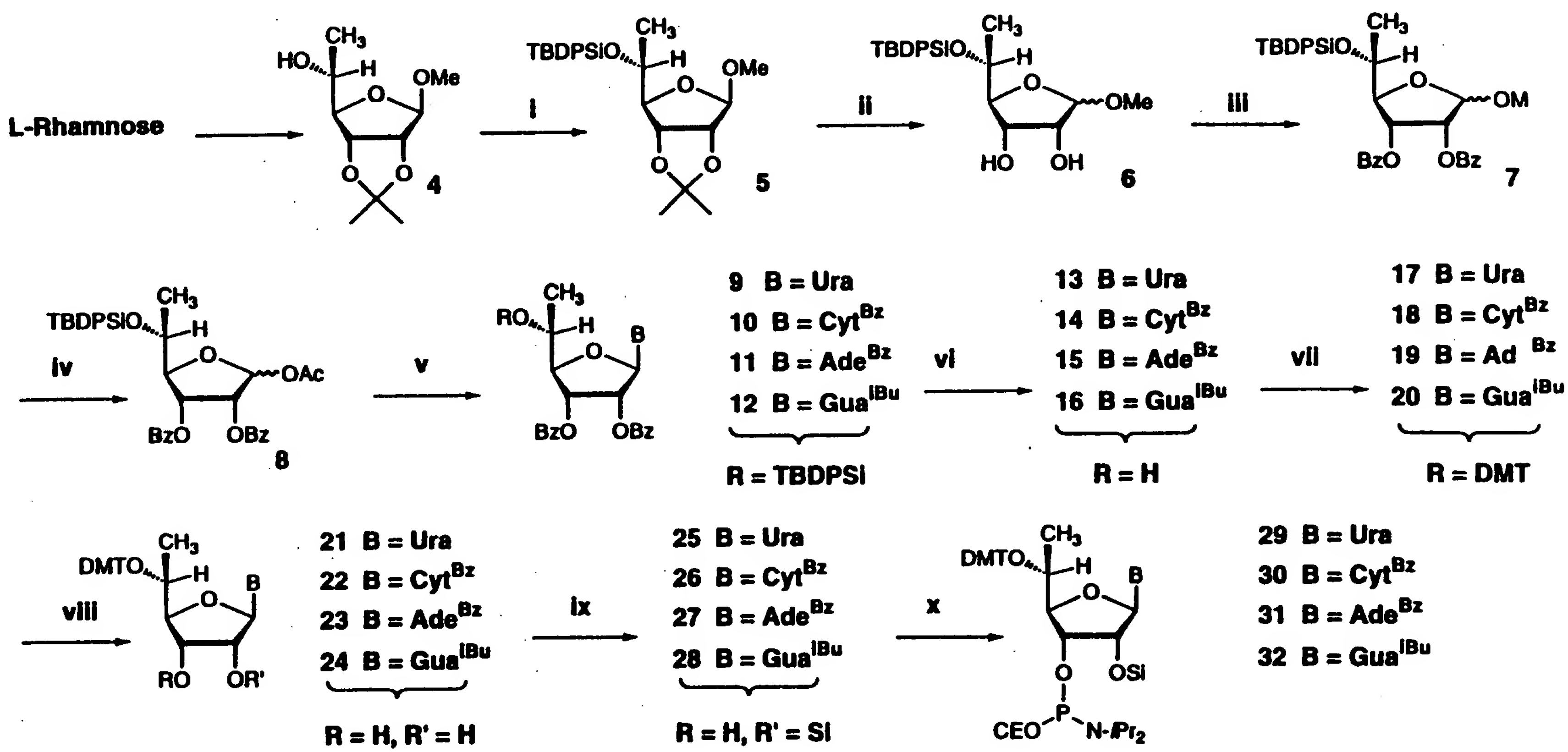
FIG. 75d. D-Allose



58-61

FIG. 75e. L-Talose

B = Protected A, C, G, U, T, 2AP, I, DiAP, P *etc.*



i) = TBDPSi-Cl
ii) = H^+
iii) = Bz-Cl/Pyr
iv) = $AcOH/Ac_2O/H^+$
v) = $B^{TMS}/CF_3SO_3SiMe_3$
vi) = TBAF
vii) = $DMT\text{-}Cl/AgNO_3$
viii) = OH^-
ix) = TBDMSi-Cl
x) = $P(OCE)(N\text{-}iPr_2)Cl$

FIG. 76.

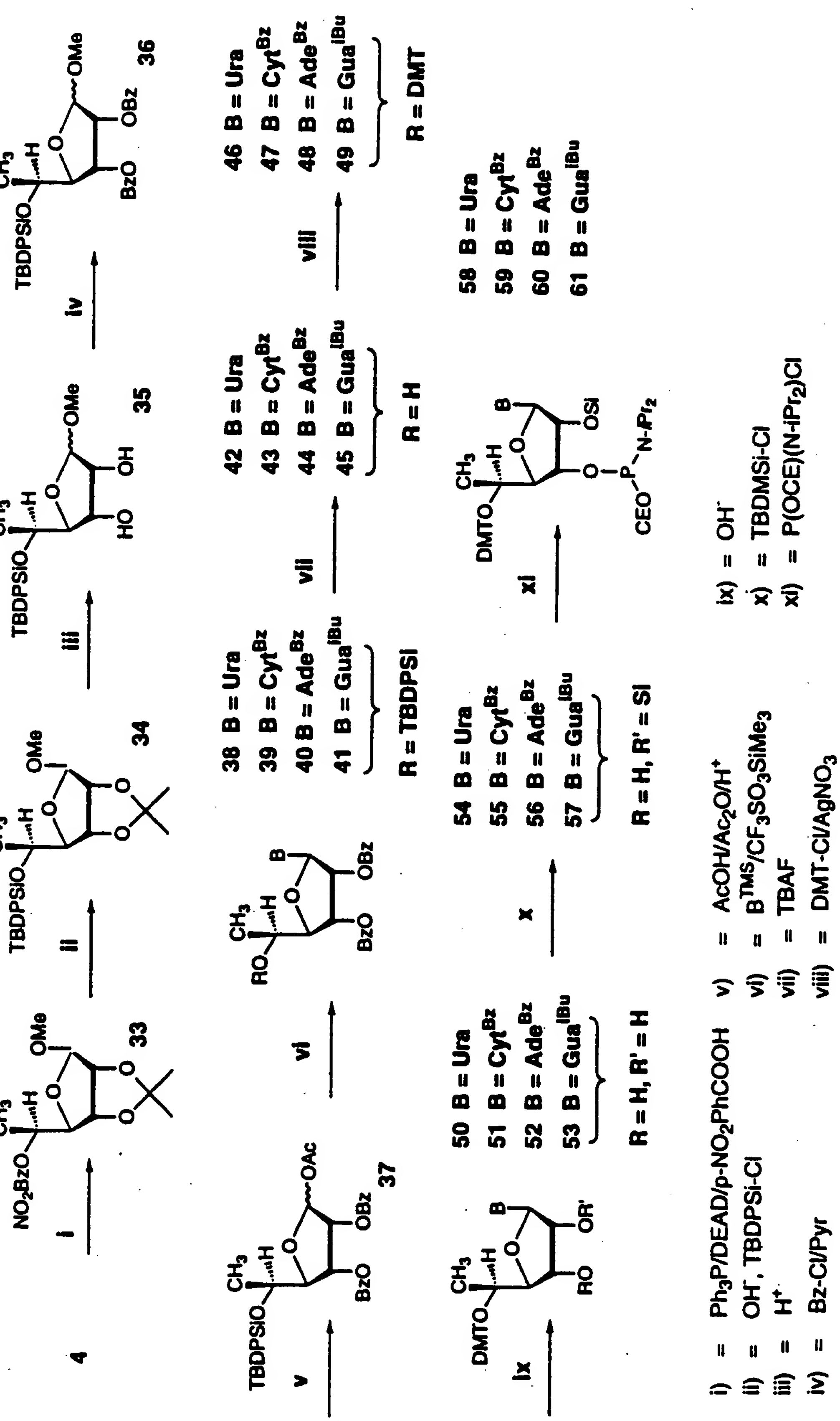
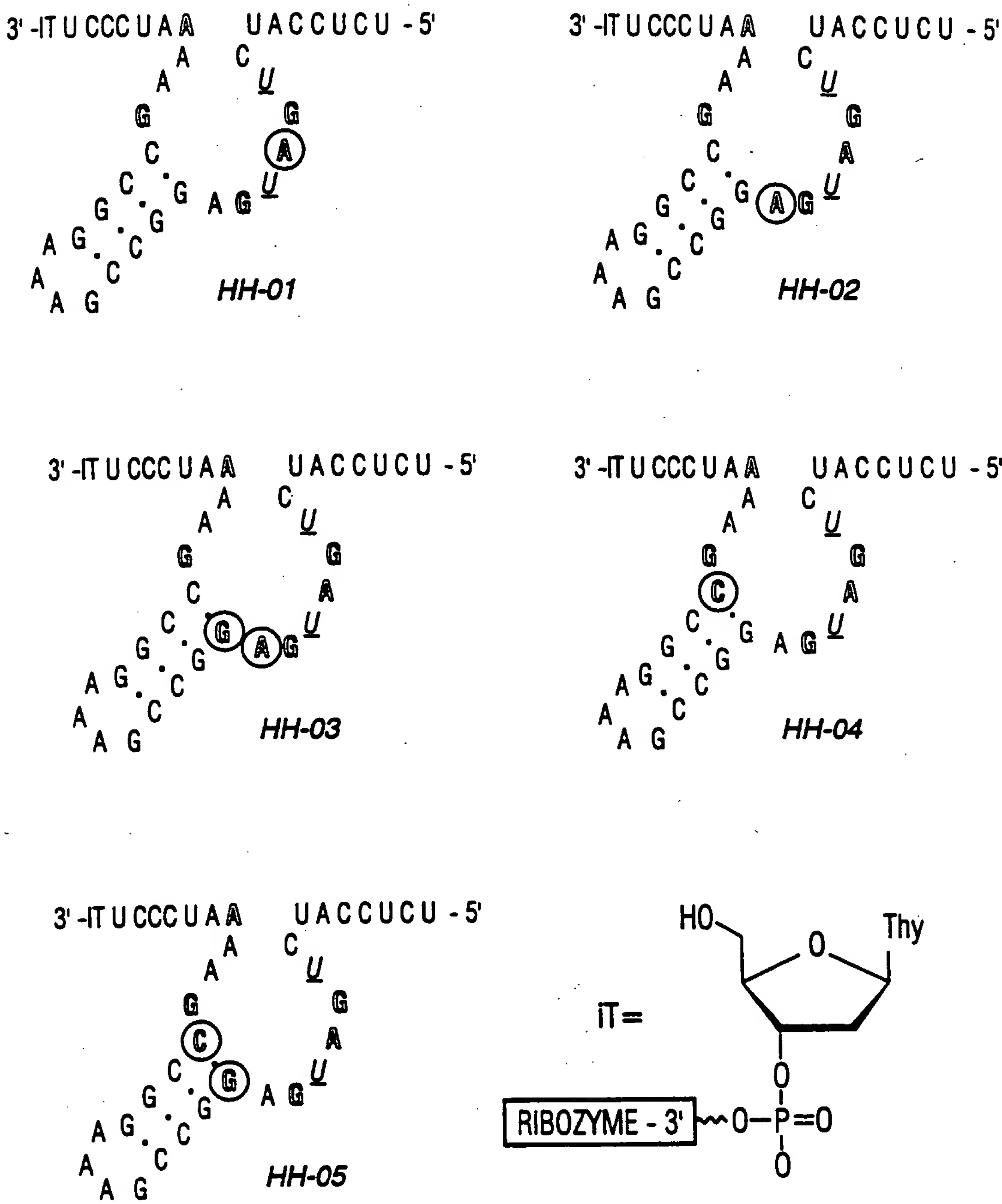

i) = Ph3P/DEAD/p-NO2PhCOOH
ii) = OH-, TBDPSi-Cl
iii) = H+
iv) = Bz-Cl/Pyr
v) = AcOH/Ac2O/H+
vi) = BTMS/CF3SO3SiMe3
vii) = TBAF
viii) = DMT-Cl/AgNO3
ix) = OH-
x) = TBDMSi-Cl
xi) = P(OCE)(N-iPr2)Cl
38 B = Ura
39 B = CytBz
40 B = AdeBz
41 B = GuaIBu
R = TBDPSi
42 B = Ura
43 B = CytBz
44 B = AdeBz
45 B = GuaIBu
R = H
46 B = Ura
47 B = CytBz
48 B = AdeBz
49 B = GuaIBu
R = DMT
50 B = Ura
51 B = CytBz
52 B = AdeBz
53 B = GuaIBu
R = H, R' = H
54 B = Ura
55 B = CytBz
56 B = AdeBz
57 B = GuaIBu
R = H, R' = Si
58 B = Ura
59 B = CytBz
60 B = AdeBz
61 B = GuaIBu

FIG. 77.

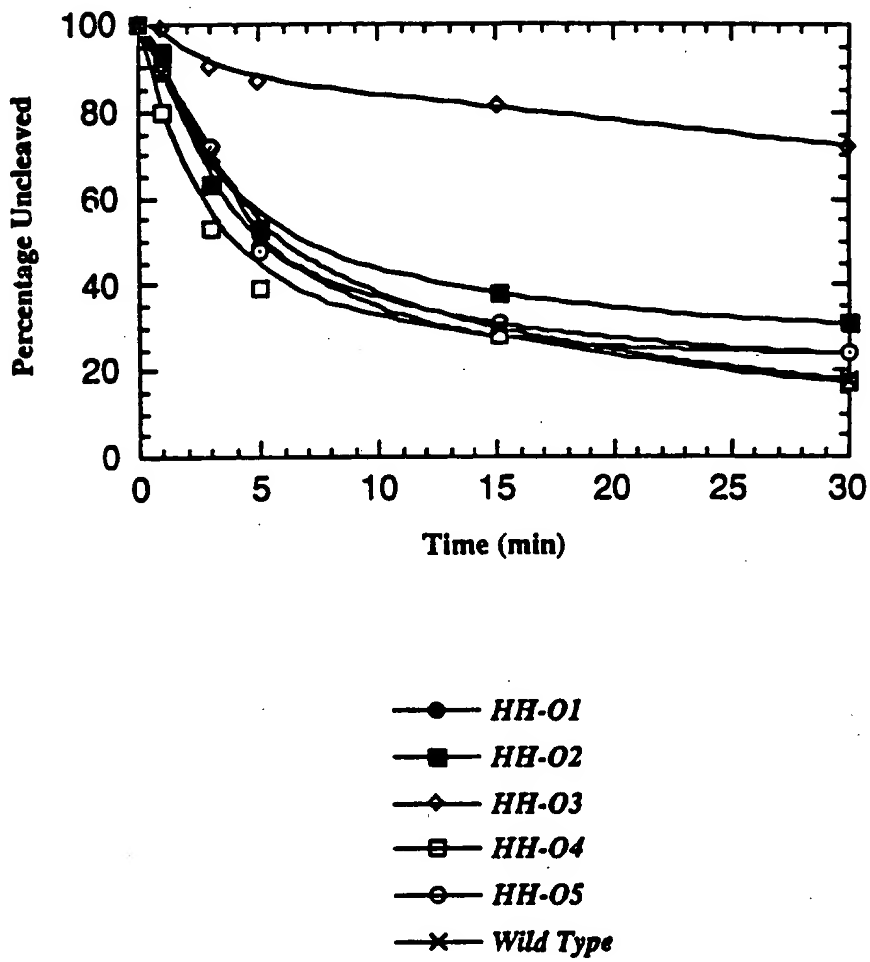
FIG. 78.



N=2'-O-Me   N =RIBO

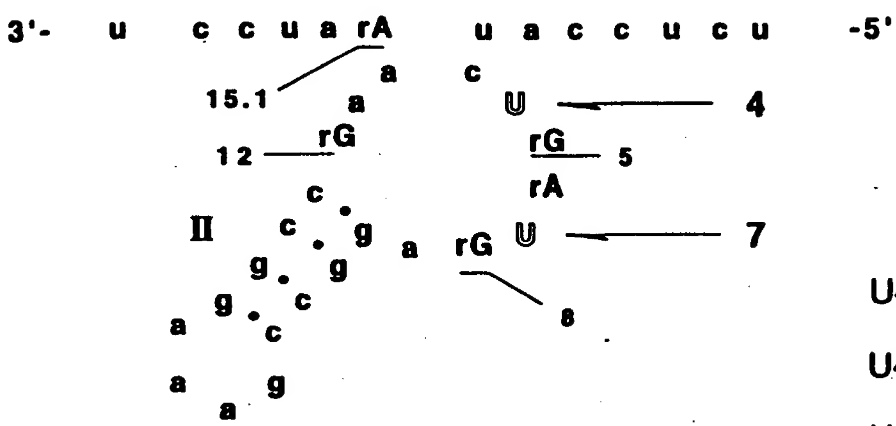
U=2'-NH_2U   (N)=TALO

WHERE THE ALPHABET "N" REPRESENTS A NUCLEOTIDE, A, U, G, OR C



100
80
60
40
20
0
Percentage Uncleaved
0
5
10
15
20
25
30
Time (min)
HH-01
HH-02
HH-03
HH-04
HH-05
Wild Type

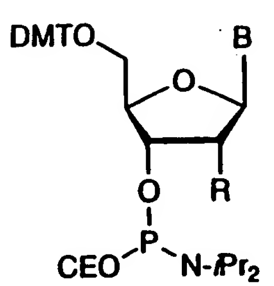
FIG. 79.



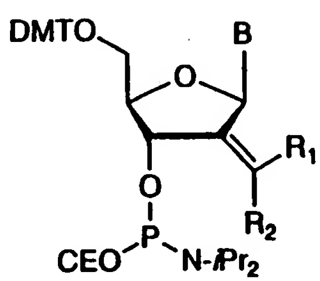
Lower case = 2'-*O*-Me
rN = ribonucleotide

		Table 1 Entries
U4 & U7	= 2'-C-Allyl-U	12-14
U4 & U7	= 2'-F-ribo-U	9-11
U4 & U7	= 2'=CH_2-U	3-5
U4 & U7	= 2'=CF_2-U	6-8
U4 & U7	= 2'-dU	21-22
U4 & U7	= 2'-F-ara-U	15-17
U4 & U7	= 2'-NH_2-U	18-20
U4 & U7	= 2'-O-Me-ribo-U	2

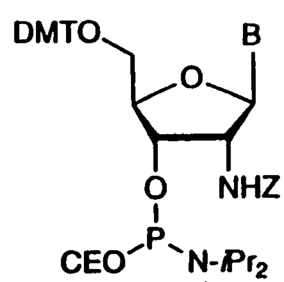
FIG. 80.



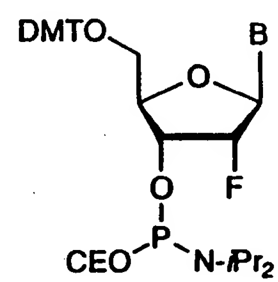
1
FIG. 81a.



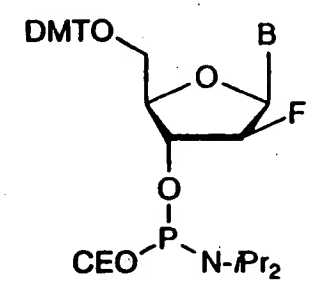
2
FIG. 81b.



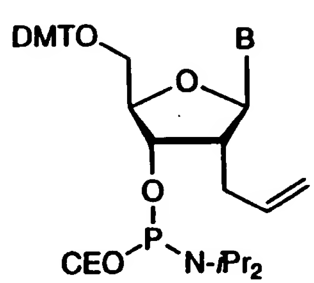
3
FIG. 81c.



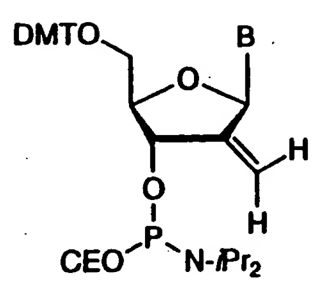
4
FIG. 81d.



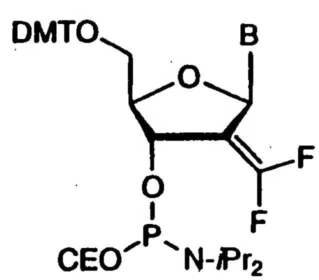
5
FIG. 81e.



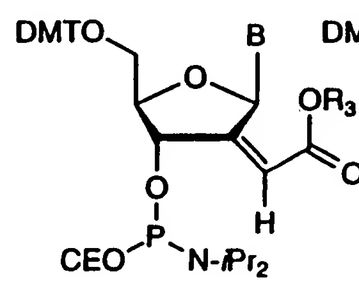
10 & 12
FIG. 81f.



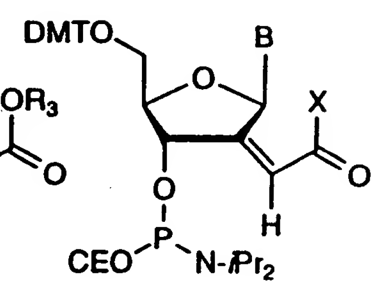
17, 22 & 31
FIG. 81g.



18, 26 & 32
FIG. 81h.

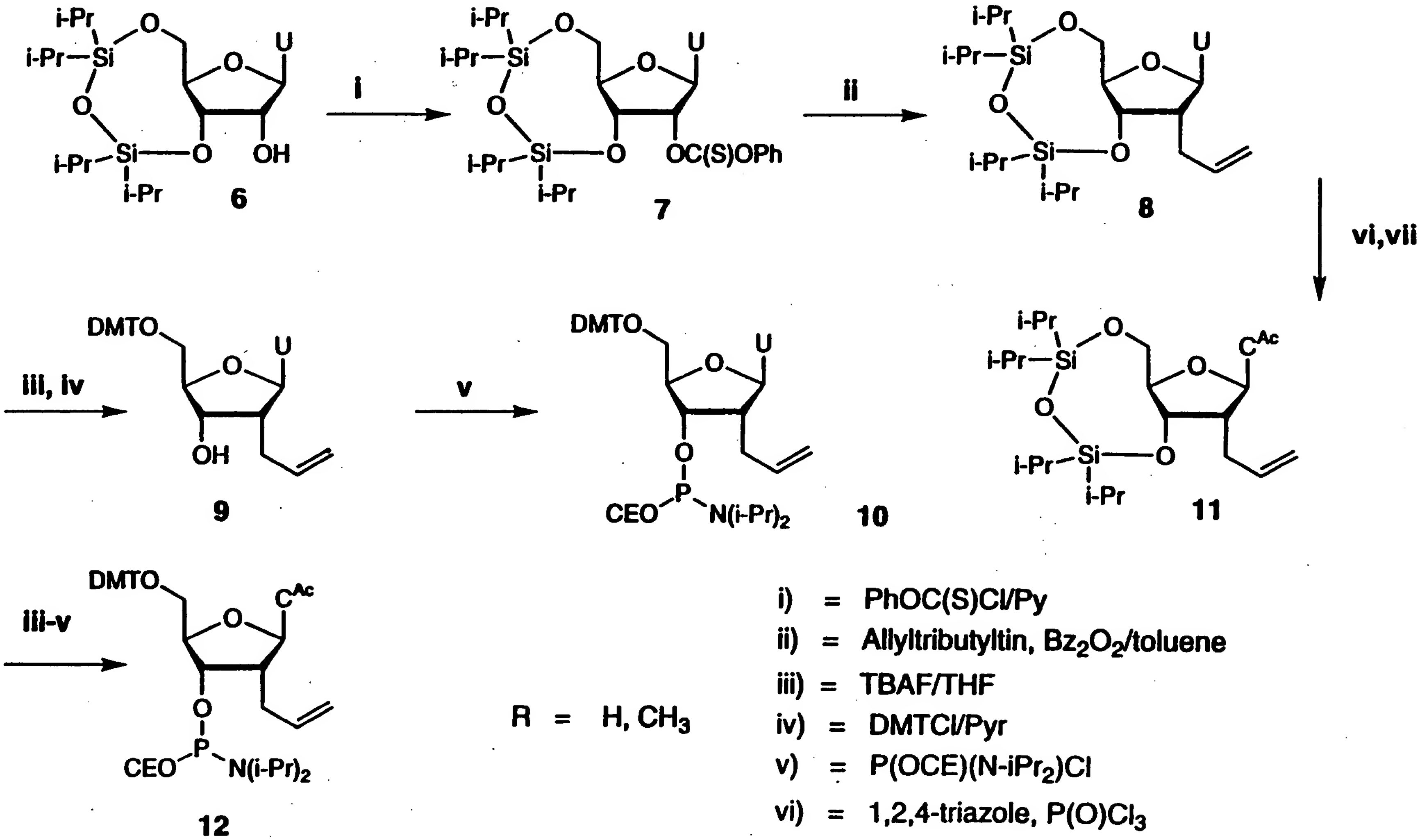


36
FIG. 81i.



38
FIG. 81j.

B = Protected A, C, G, U, T, 2AP, I, DiAP, P *etc.*

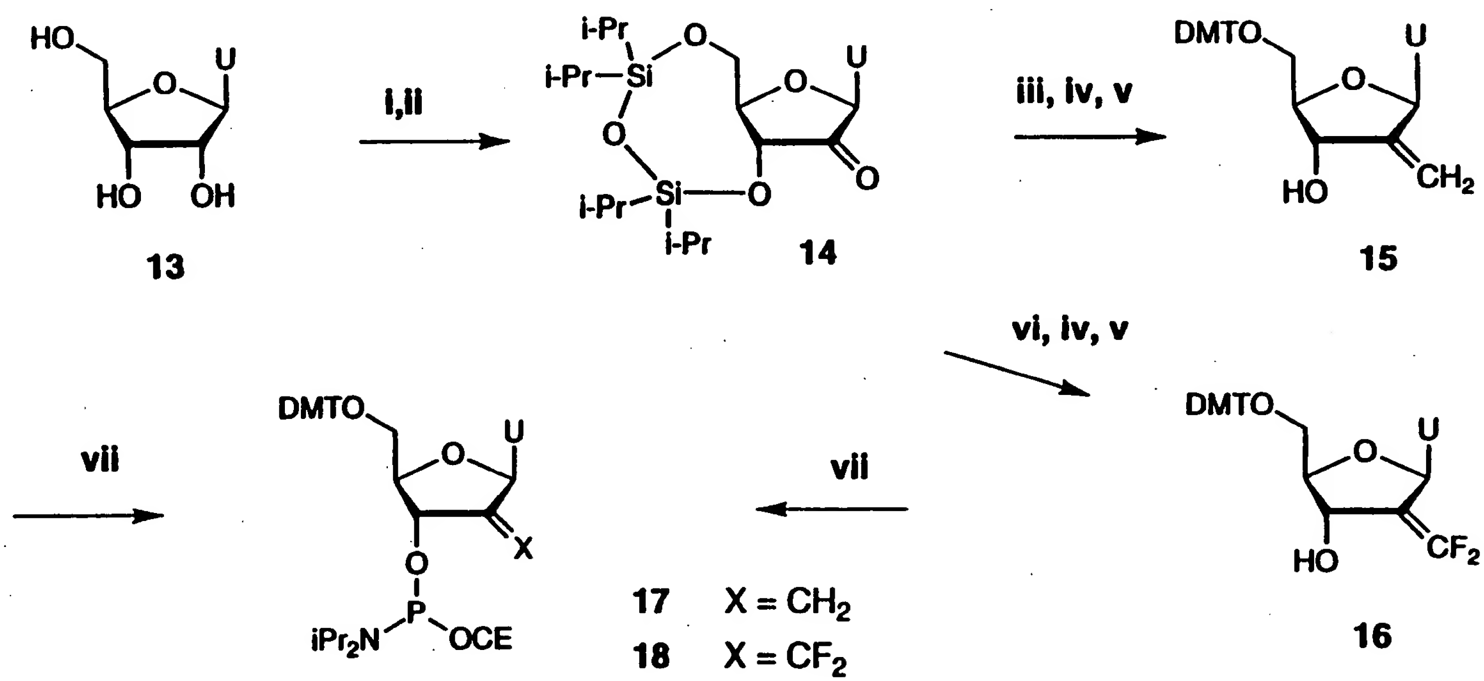


R = H, CH_3

i) = PhOC(S)Cl/Py
ii) = Allyltributyltin, Bz_2O_2/toluene
iii) = TBAF/THF
iv) = DMTCl/Pyr
v) = P(OCE)(N-iPr_2)Cl
vi) = 1,2,4-triazole, P(O)Cl_3
vii) = 29% NH_4OH/dioxane, Ac_2O/Pyr

FIG. 82.

FIG. 83.



i) = Markiewicz reagent

ii) = DMSO & Ac_2O

iii) = Ph_3PCH_3I

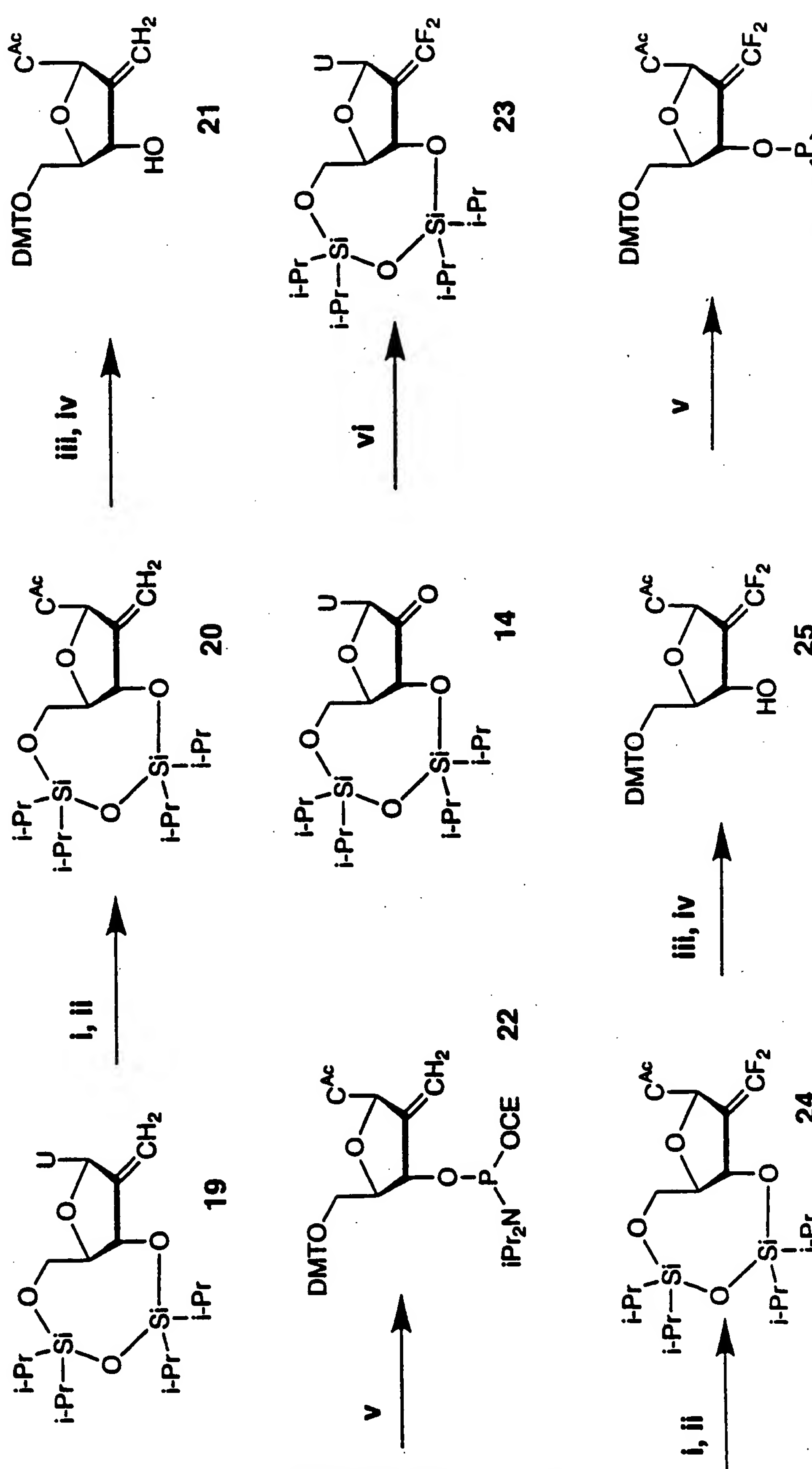
iv) = TBAF/THF

v) = DMTCl/Pyr

vi) = Ph_3P, $ClCF_2COONa$

vii) = $P(OCE)(N\text{-}iPr_2)Cl$

FIG. 84.



i) = 1,2,4-triazole, P(O)Cl_3

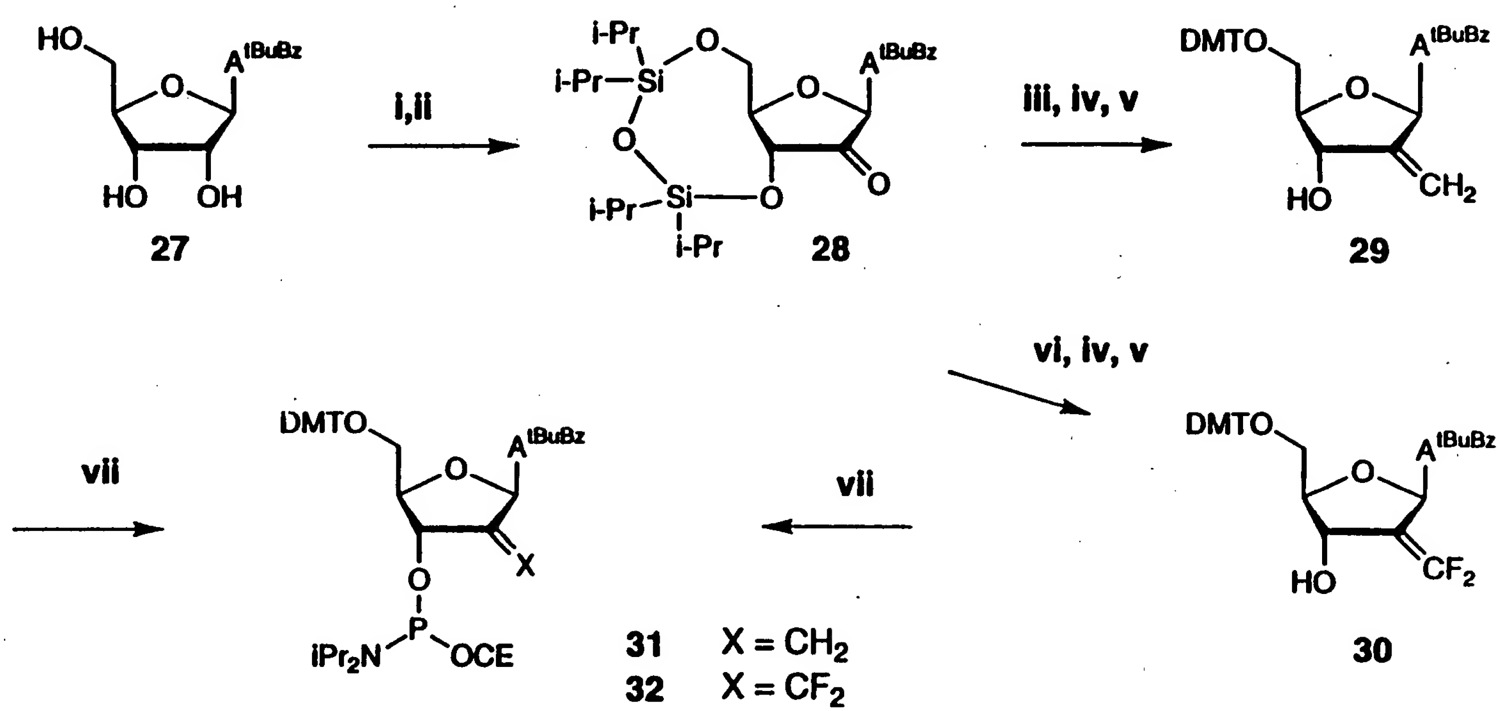
ii) = 29% NH_4OH/dioxane, Ac_2O/Pyr

iii) = TBAF/THF

iv) = DMTCl/Pyr

v) = P(OCE)(N-iPr_2)Cl

vi) = Ph_3P, $ClCF_2COONa$



i)	=	Markiewicz reagent
ii)	=	DMSO & Ac_2O
iii)	=	Ph_3PCH_3I
iv)	=	TBAF/THF
v)	=	DMTCl/Pyr
vi)	=	Ph_3P, $ClCF_2COONa$
vii)	=	$P(OCE)(N\text{-}iPr_2)Cl$

FIG. 85.

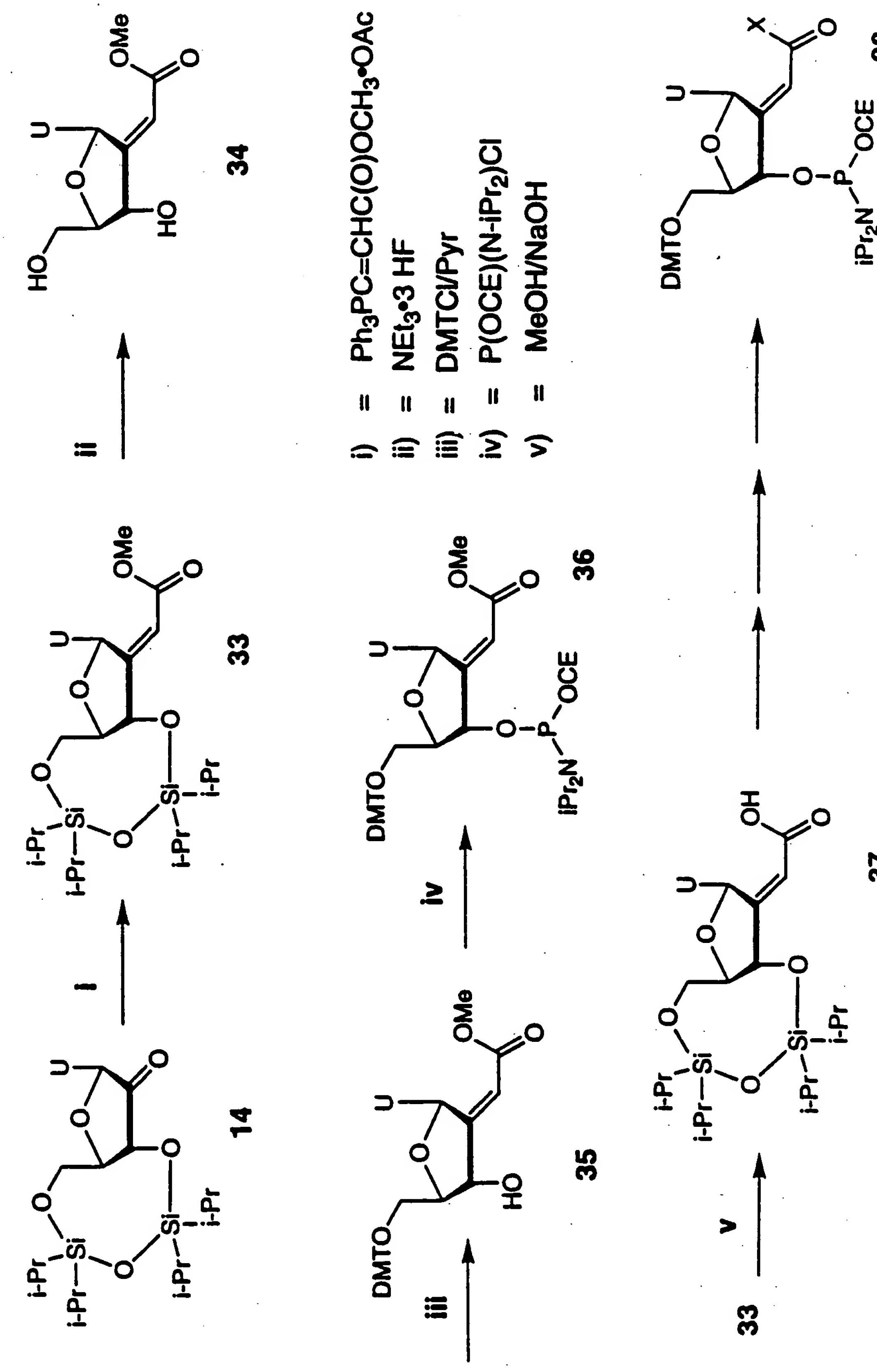
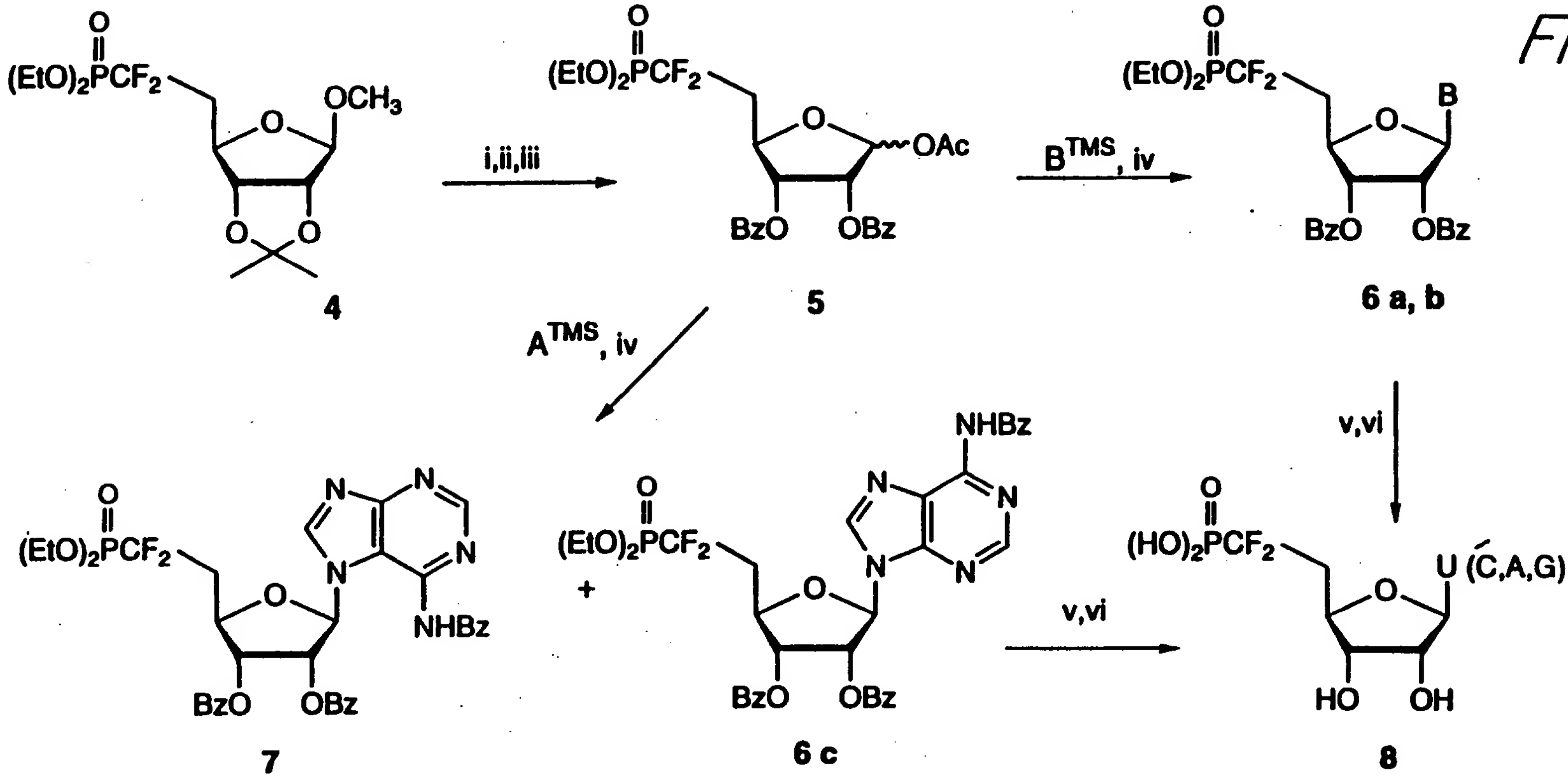
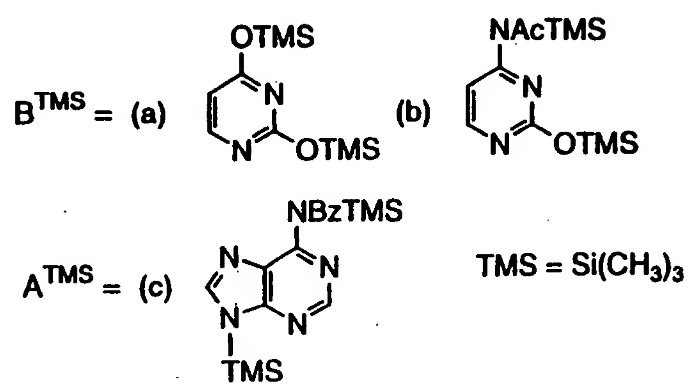

i) = $Ph_3PC{=}CHC(O)OCH_3{\bullet}OAc$
ii) = $NEt_3{\bullet}3\ HF$
iii) = DMTCl/Pyr
iv) = $P(OCE)(N\text{-}iPr_2)Cl$
v) = MeOH/NaOH

FIG. 86

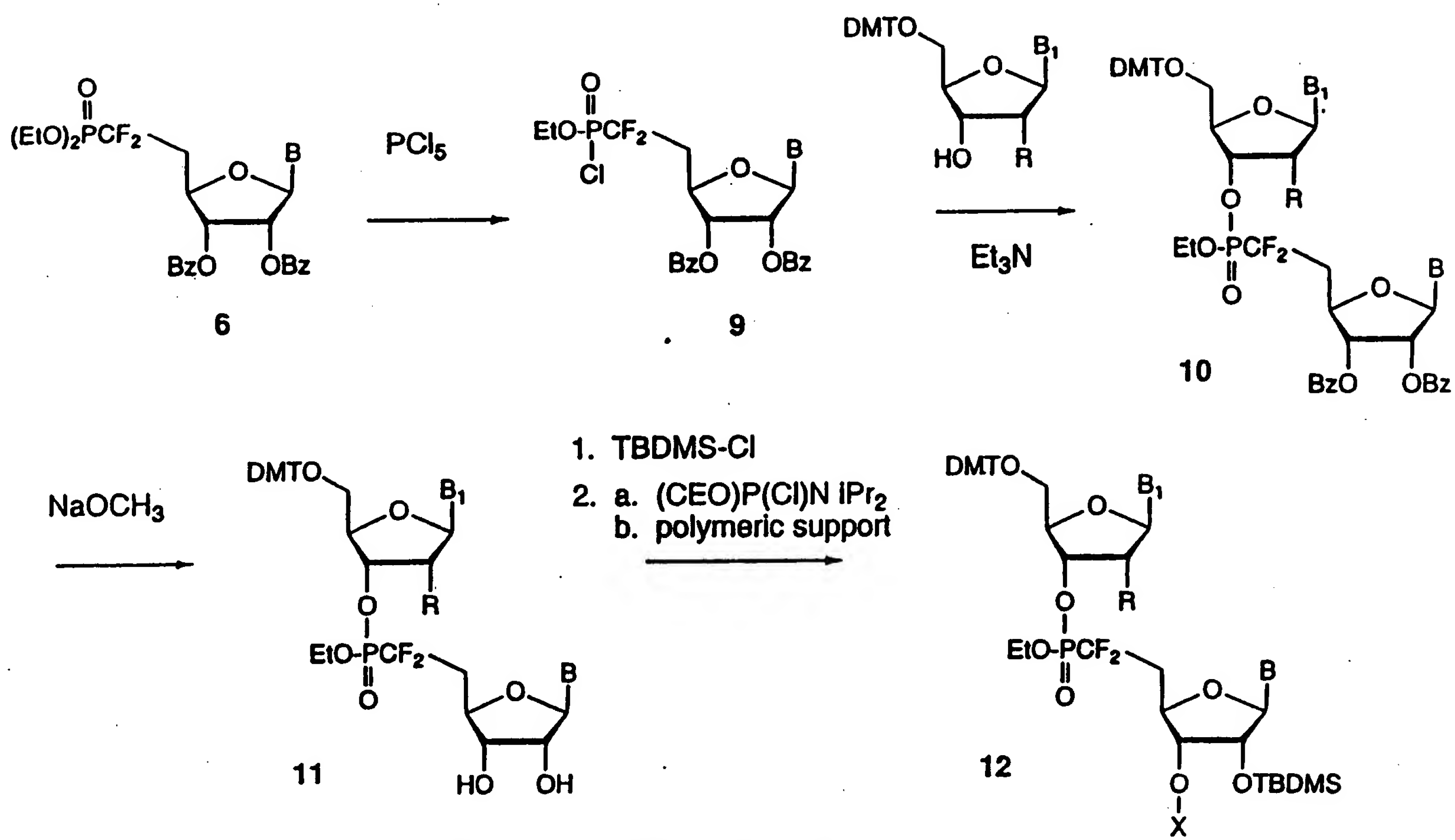
FIG. 87.





Reagents and Conditions:

i) I_2-MeOH, reflux, 18 h or Dowex 50 WX8 (H^+), MeOH, RT, 3 days
ii) BzCl, Py, RT, 16 h
iii) Ac_2O, AcOH, H_2SO_4, EtOAc, 0 °C, 18 h
iv) $SnCl_4$, CH_3CN, reflux, 2 h
v) $(CH_3)_3SiBr$, DMF, RT, 72 h
vi) conc. NH_4OH-MeOH (3:1), 60 °C, 18 h



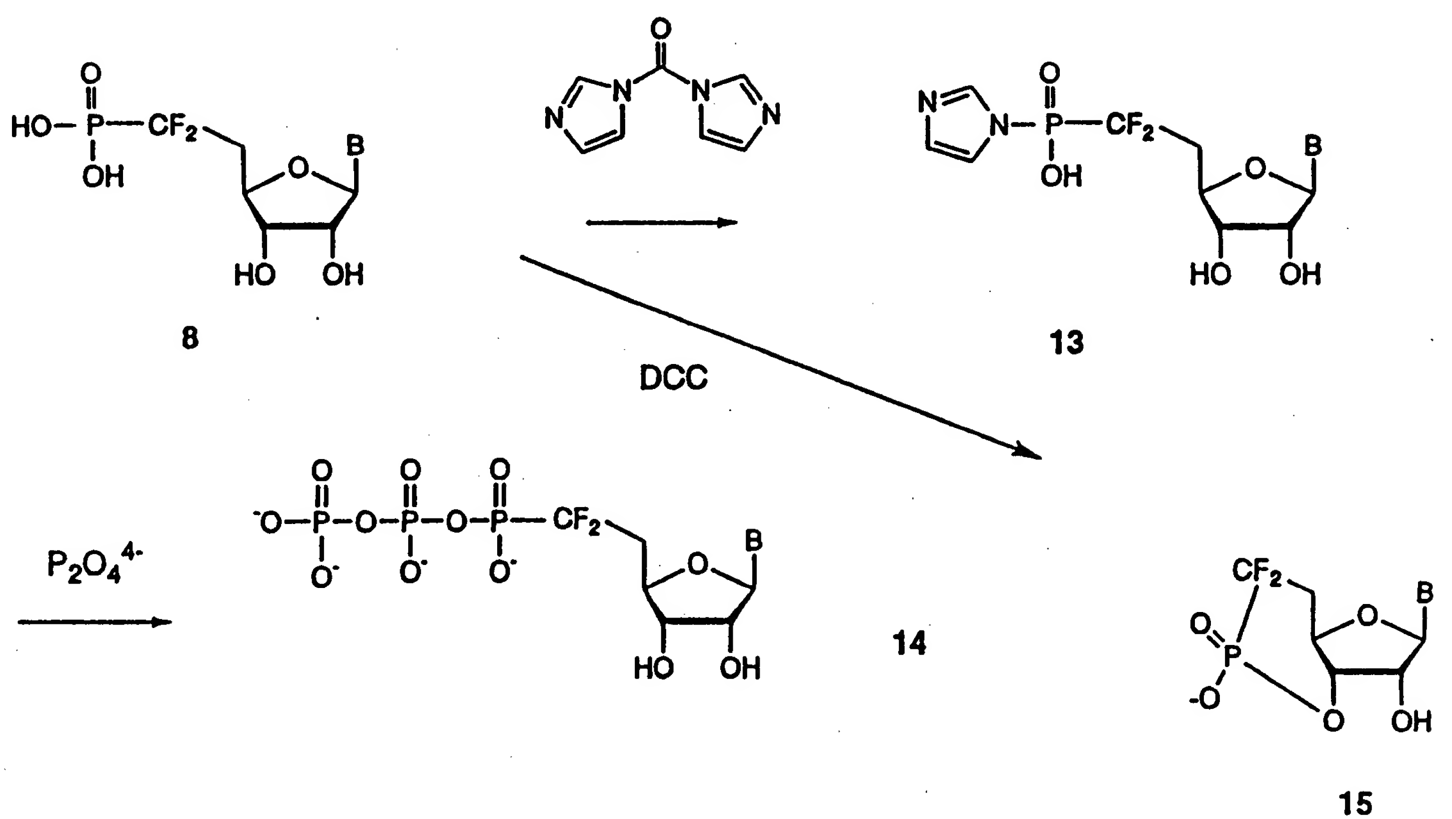
B, B_1 = uracil, *N*-Z-cytosine, *N*-Z-adenine, *N*-Z-guanine *etc.*

Z = amino-protecting group

R = OTBDMS, OCH_3, H

a. X = P(OCE)N iPr_2
X = polymeric support

FIG. 88.



B = uracil, *N*-Z-cytosine, *N*-Z-adenine, *N*-Z-guanine *etc.*

Z = amino-protecting group

FIG. 89.

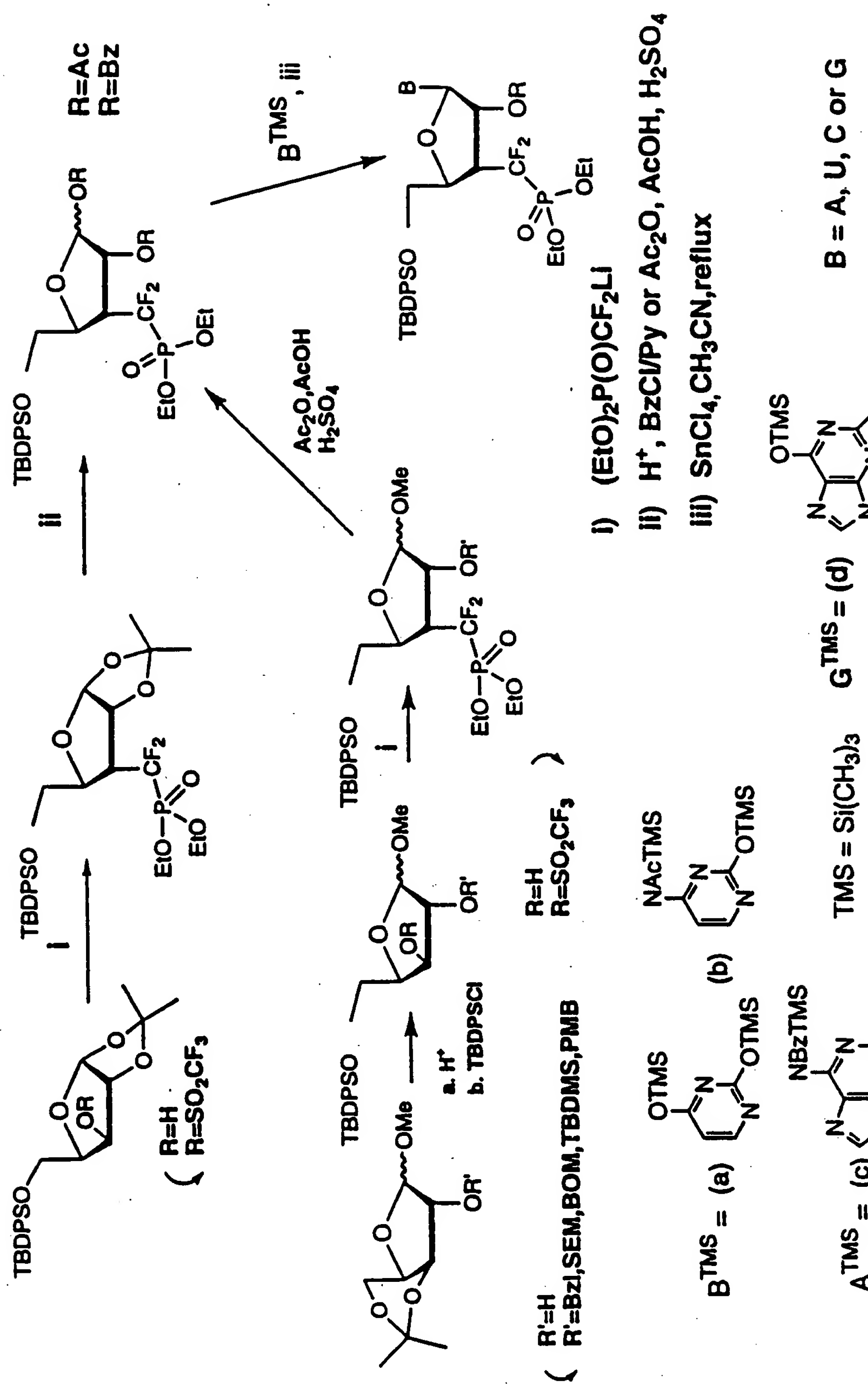

TBDPSO
R=H
R=SO2CF3
i
ii
R=Ac
R=Bz
B^TMS, iii
Ac2O,AcOH
H2SO4
a. H+
b. TBDPSCl
R'=H
R'=Bzl,SEM,BOM,TBDMS,PMB
i) (EtO)2P(O)CF2Li
ii) H+, BzCl/Py or Ac2O, AcOH, H2SO4
iii) SnCl4,CH3CN,reflux
B = A, U, C or G
B^TMS = (a)
(b)
A^TMS = (c)
G^TMS = (d)
TMS = Si(CH3)3

FIG. 90.

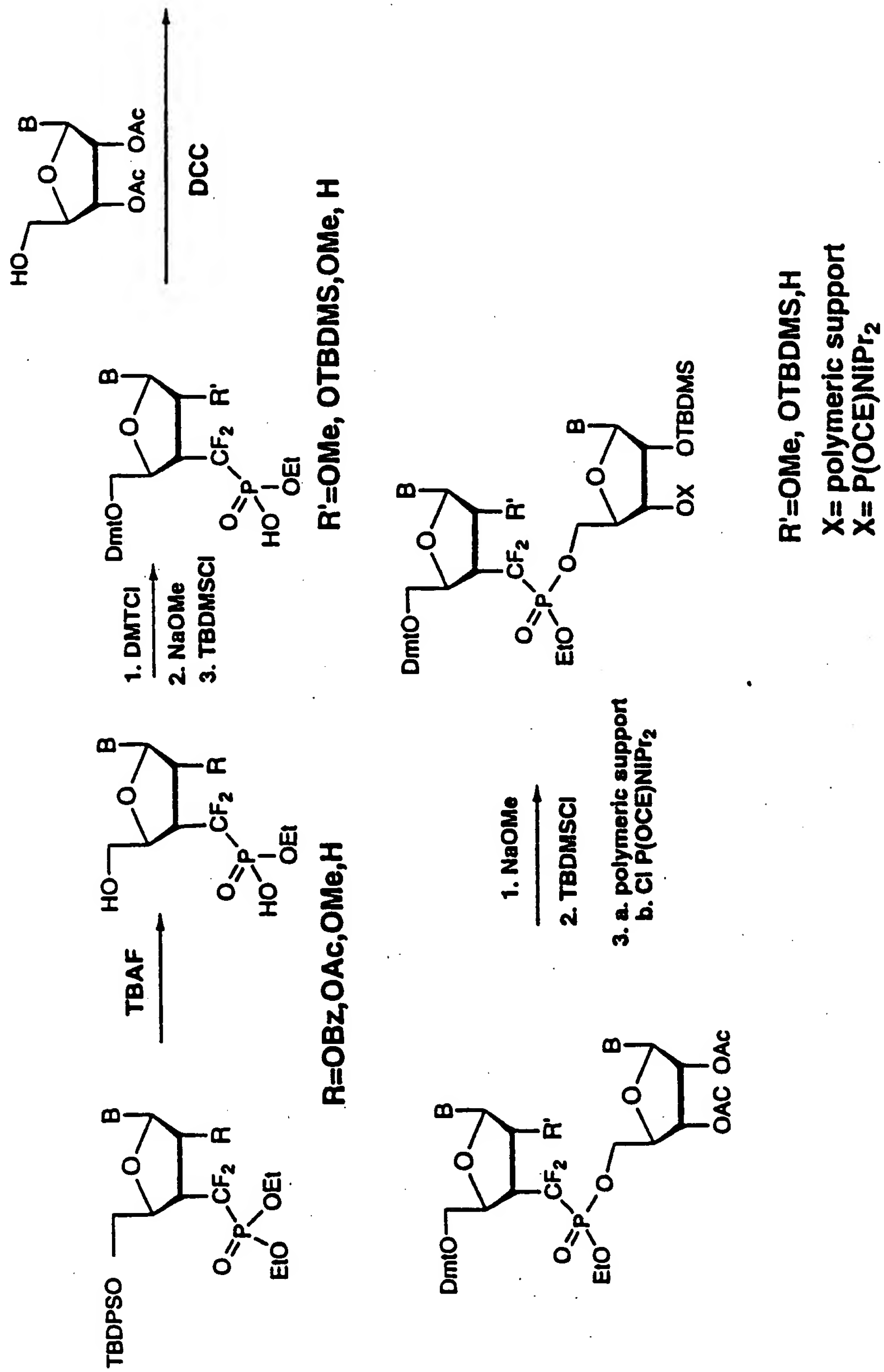

TBDPSO
EtO
OEt
CF2
R
B
TBAF
HO
HO
OEt
R=OBz,OAc,OMe,H
1. DMTCl
2. NaOMe
3. TBDMSCl
DmtO
R'
R'=OMe, OTBDMS,OMe, H
DCC
OAc
1. NaOMe
2. TBDMSCl
3. a. polymeric support
b. Cl P(OCE)NiPr2
OTBDMS
OX
R'=OMe, OTBDMS,H
X= polymeric support
X= P(OCE)NiPr2

FIG. 91.

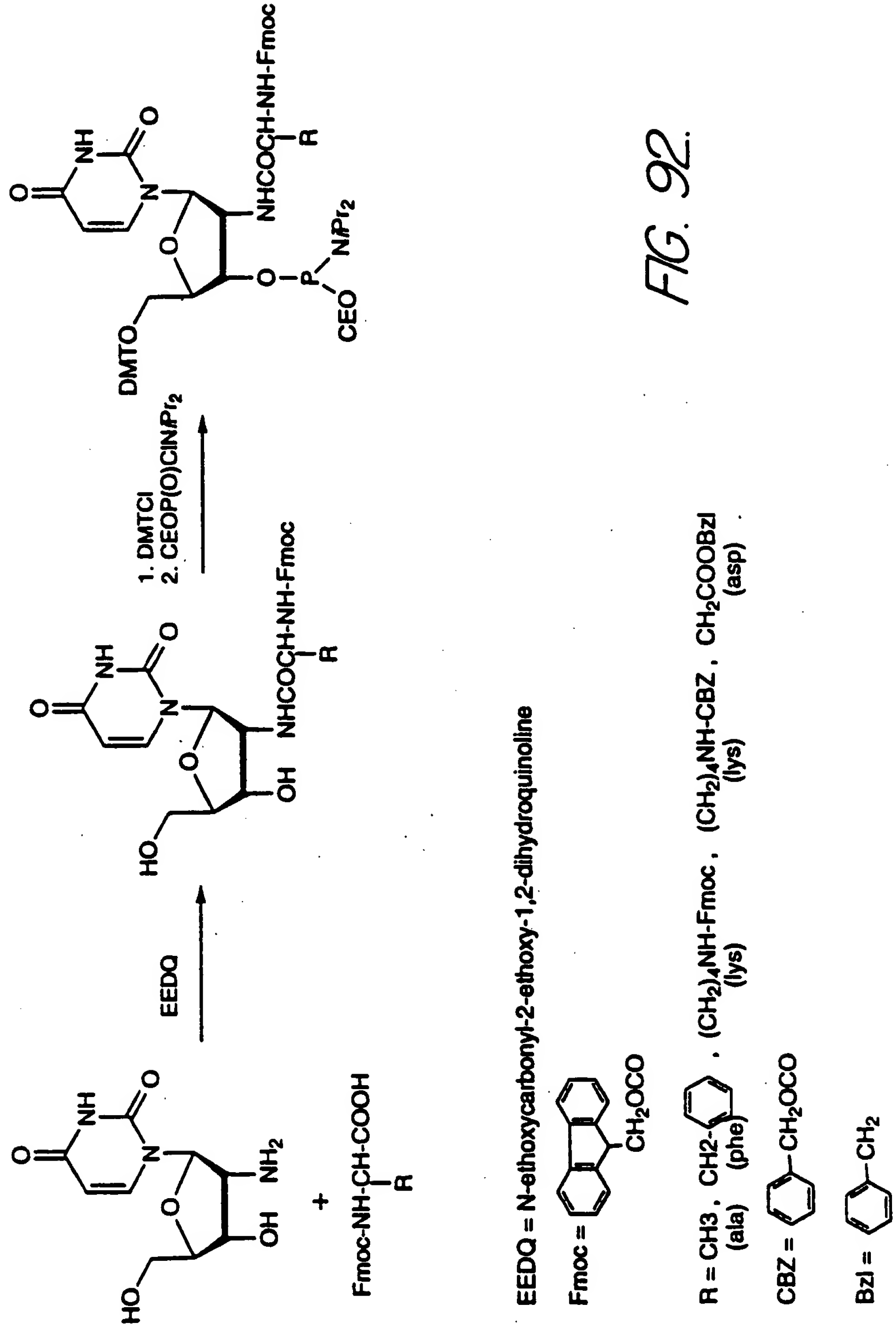

EEDQ
1. DMTCl
2. CEOP(O)ClNiPr2
HO
OH
NH2
Fmoc-NH-CH-COOH
R
NHCOCH-NH-Fmoc
DMTO
CEO
NiPr2
EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
Fmoc =
CH2OCO
R = CH3 , CH2- , (CH2)4NH-Fmoc , (CH2)4NH-CBZ , CH2COOBzl
(ala) (phe) (lys) (lys) (asp)
CBZ =
CH2OCO
Bzl =
CH2

FIG. 92.

FIG. 93a.

A

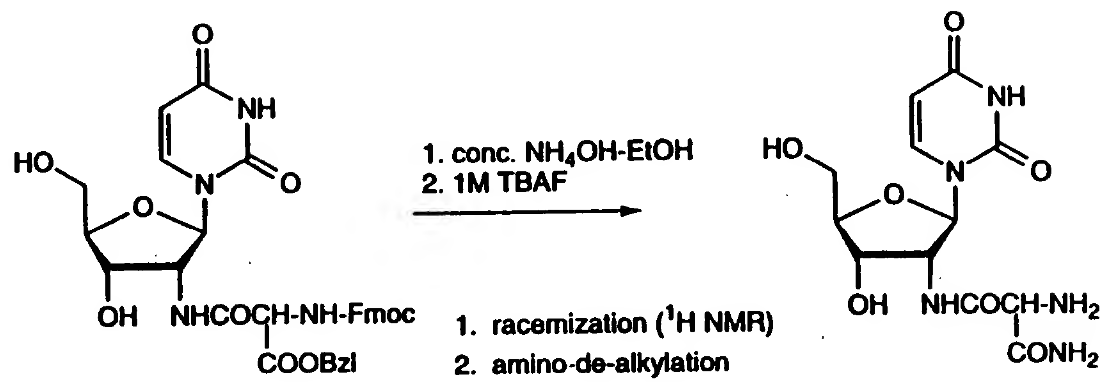
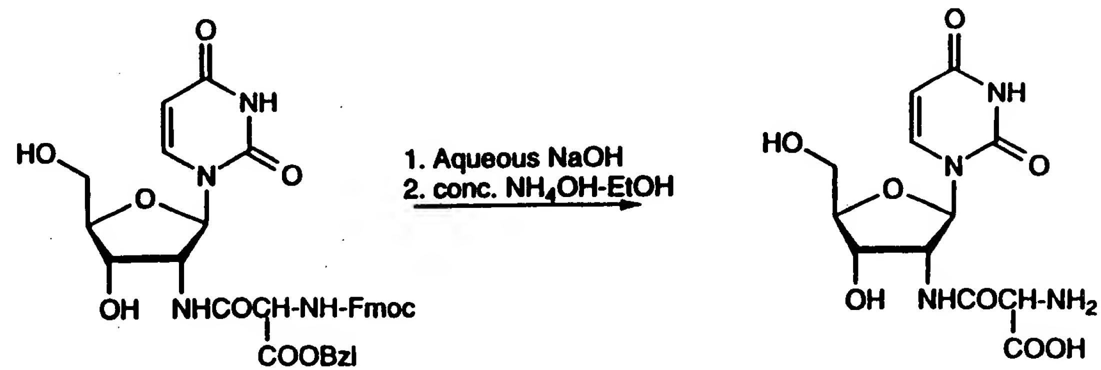


FIG. 93b.

B



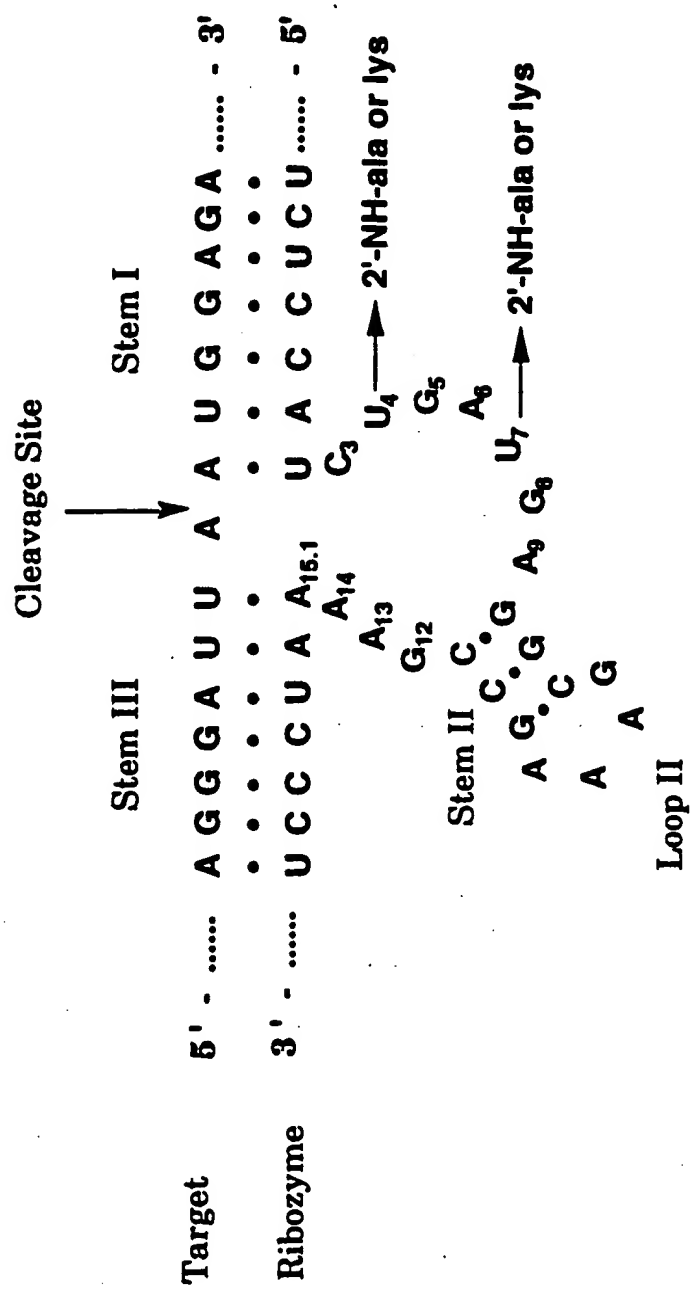
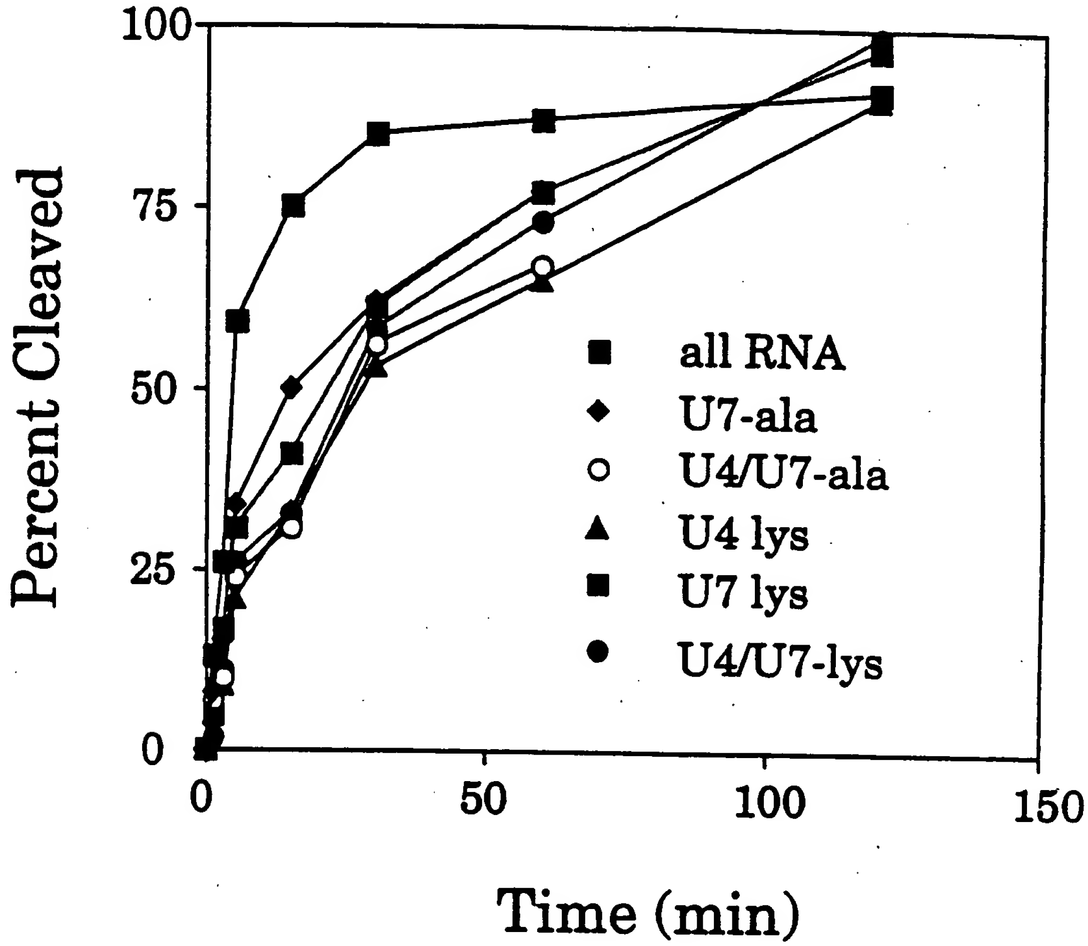

Cleavage Site
Stem III
Stem I
Target
5' - ...... A G G G A U U A A U G G A G A ...... - 3'
Ribozyme
3' - ...... U C C C U A U U A C C U C U ...... - 5'
$A_{15.1}$
A_{14}
A_{13}
G_{12}
C_3
U_4
2'-NH-ala or lys
G_5
A_6
U_7
2'-NH-ala or lys
G_8
A_9
Stem II
Loop II

FIG. 94.



[Ribozyme] = 40 nM [Substrate] = ~1nM

FIG. 95.

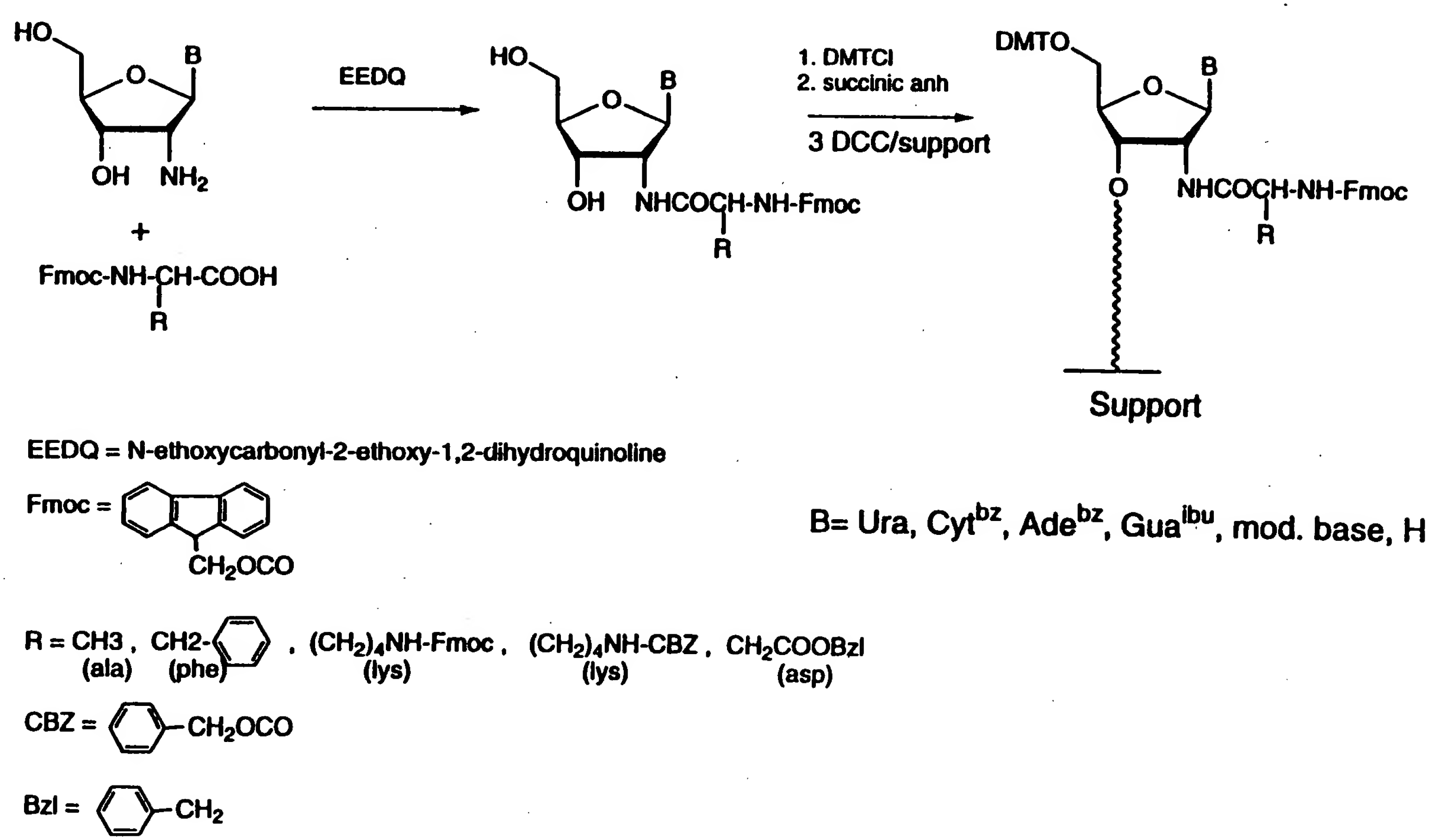

HO
B
O
OH NH2
+
Fmoc-NH-CH-COOH
R
EEDQ
HO
B
O
OH NHCOCH-NH-Fmoc
R
1. DMTCl
2. succinic anh
3 DCC/support
DMTO
B
O
O
NHCOCH-NH-Fmoc
R
Support
EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
Fmoc =
CH2OCO
B= Ura, Cytbz, Adebz, Guaibu, mod. base, H
R = CH3 , CH2- , (CH2)4NH-Fmoc , (CH2)4NH-CBZ , CH2COOBzl
(ala) (phe) (lys) (lys) (asp)
CBZ = -CH2OCO
Bzl = -CH2

FIG. 96.

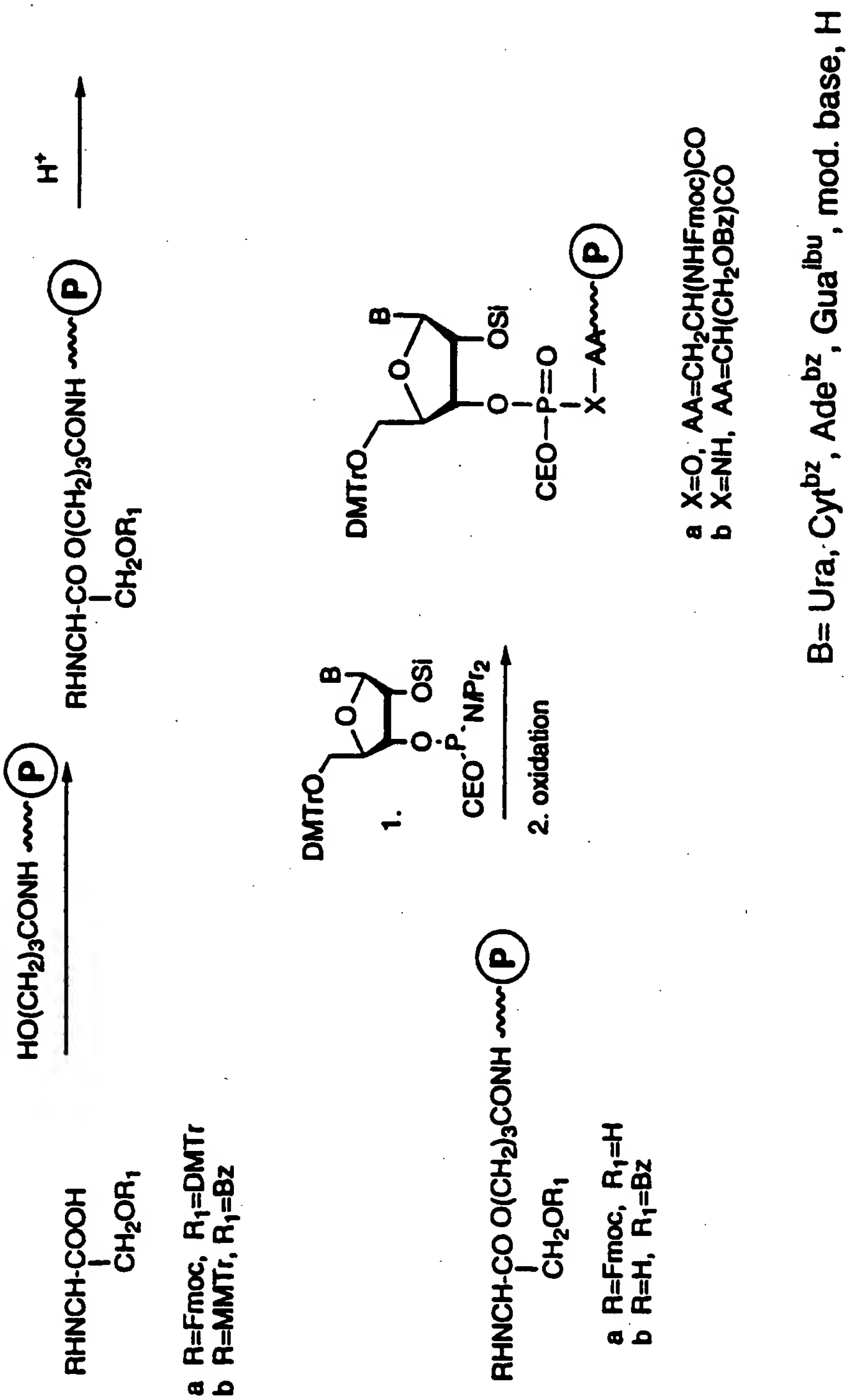
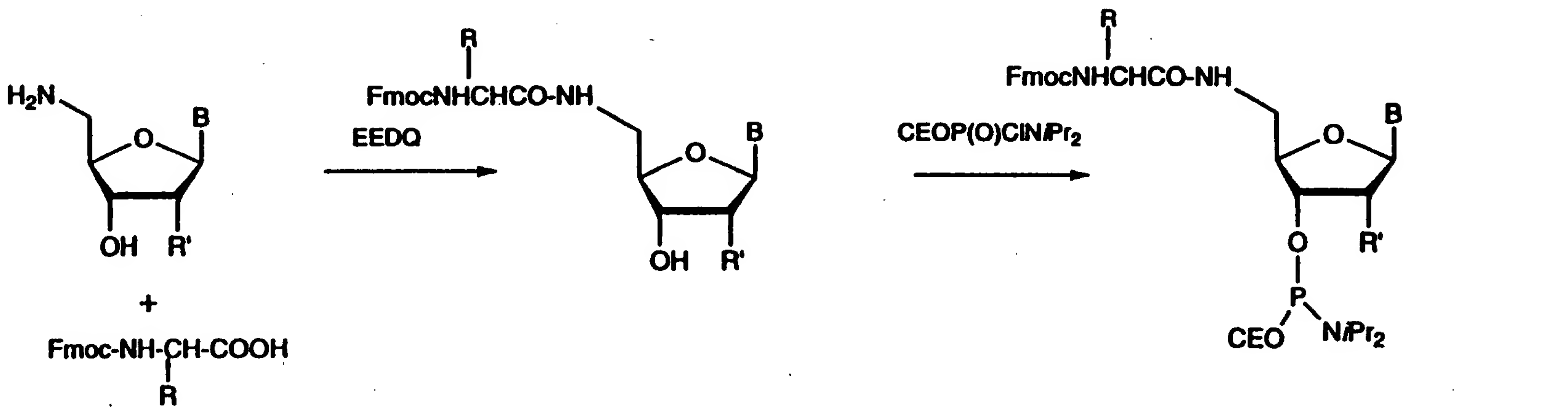
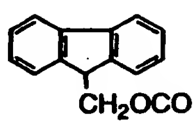

RHNCH-COOH
CH2OR1
a R=Fmoc, R1=DMTr
b R=MMTr, R1=Bz
HO(CH2)3CONH ~ P
RHNCH-CO O(CH2)3CONH ~ P
CH2OR1
H+
RHNCH-CO O(CH2)3CONH ~ P
CH2OR1
a R=Fmoc, R1=H
b R=H, R1=Bz
DMTrO
OSi
CEO
NiPr2
2. oxidation
a X=O, AA=CH2CH(NHFmoc)CO
b X=NH, AA=CH(CH2OBz)CO
B= Ura, Cytbz, Adebz, Guaibu, mod. base, H

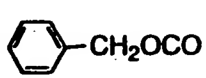
FIG. 97.

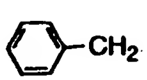


EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

Fmoc = 

R = CH3 (ala), CH2- (phe), $(CH_2)_4$NH-Fmoc (lys), $(CH_2)_4$NH-CBZ (lys), CH_2COOBzl (asp)

CBZ = 

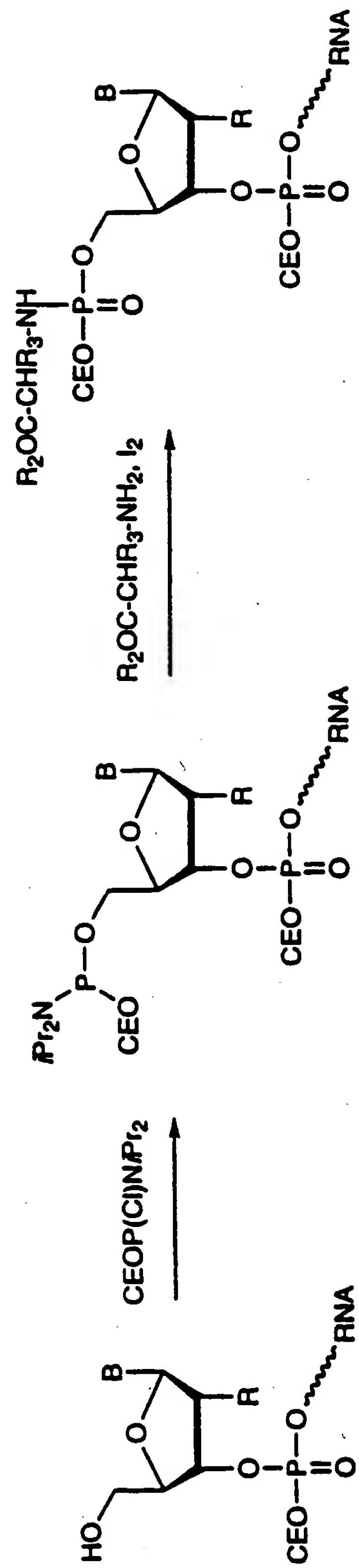
Bzl = 

R'= H,OMe, OTBDMSi

B= Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

FIG. 98.

FIG. 99.



B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

R = H, OCH_3, OTBDMS, Hal, NHR_1

R_2 = OBzl, peptidyl

FIG. 100.

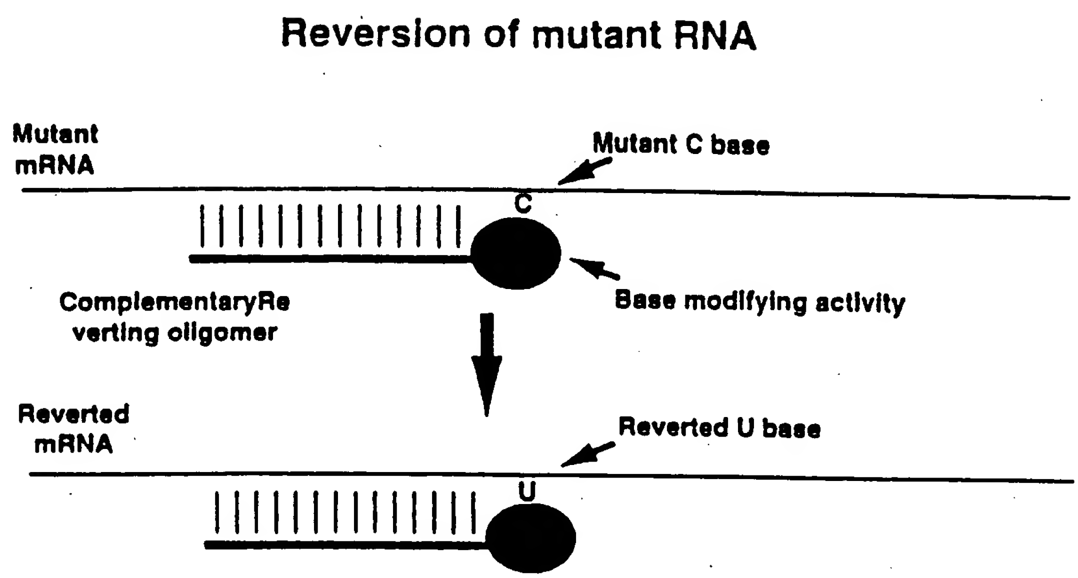
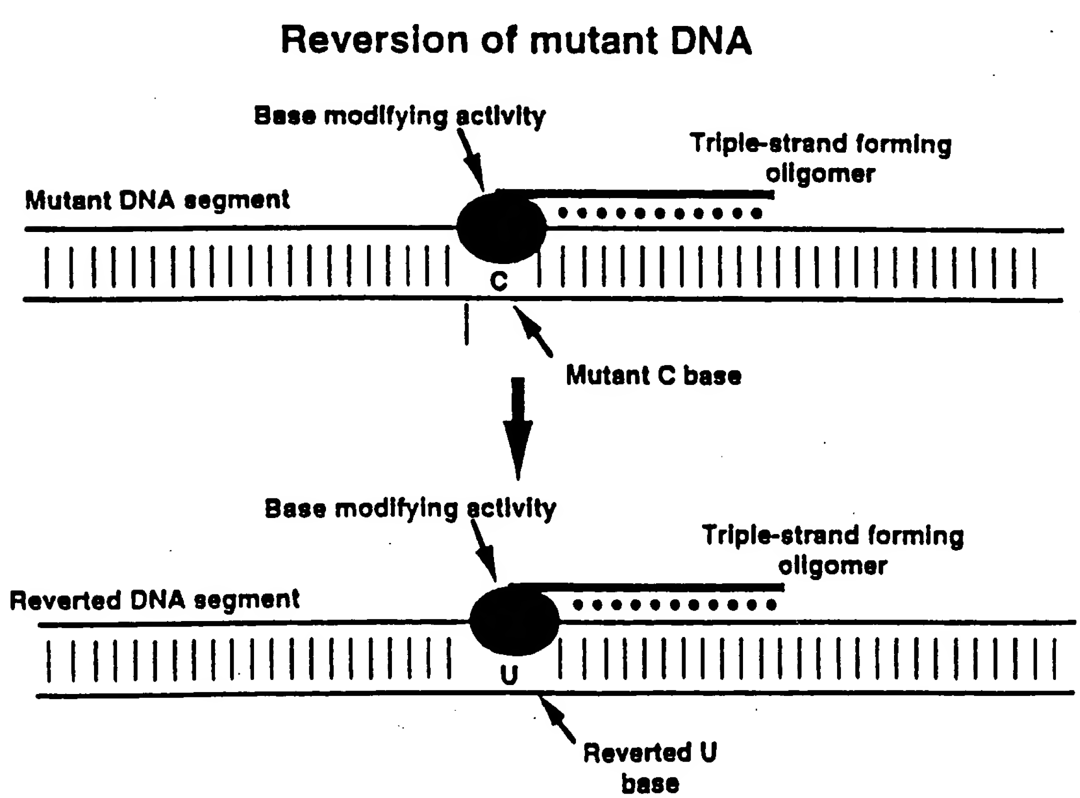


FIG. 101.



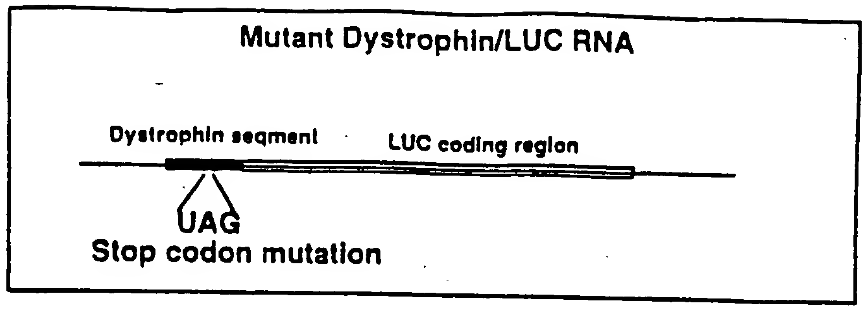


FIG. 102a.

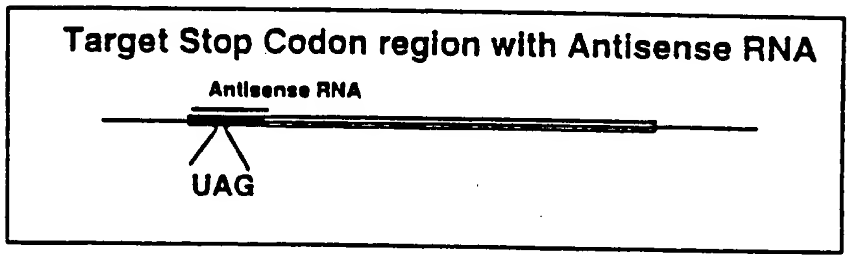


FIG. 102b.

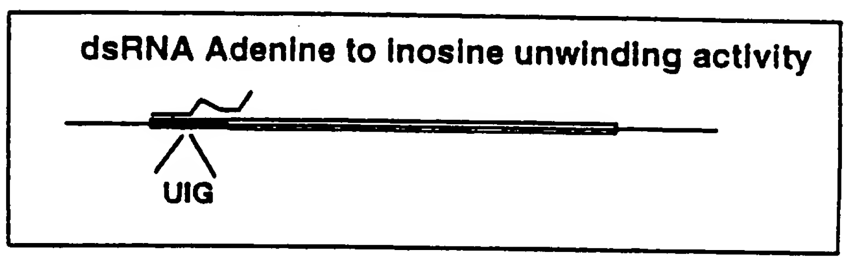


FIG. 102c.

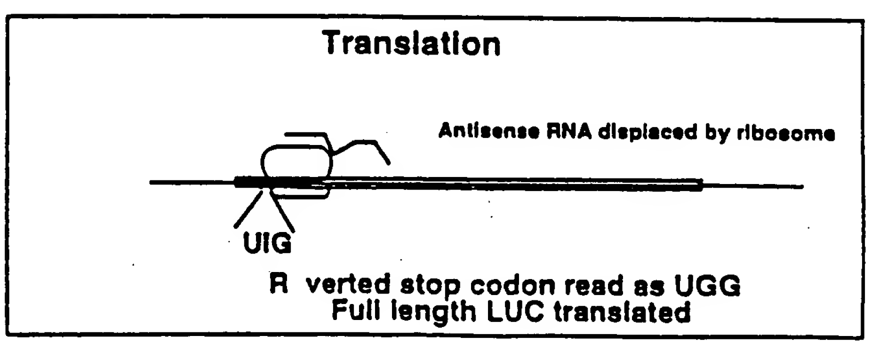
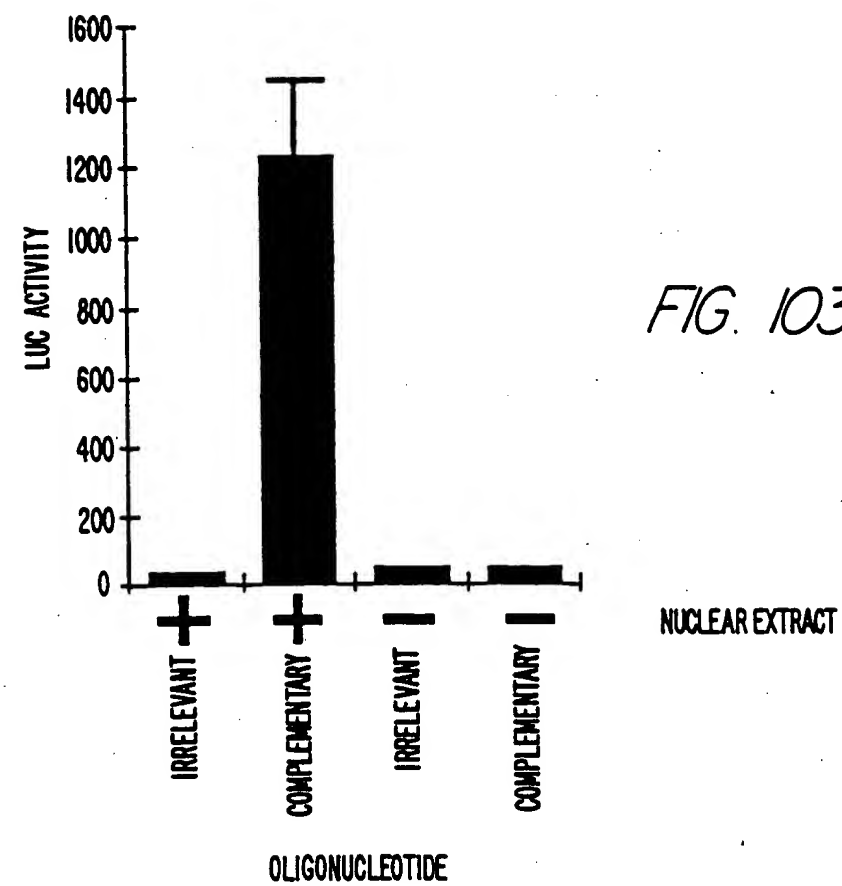


FIG. 102d.



1600
1400
1200
1000
800
600
400
200
0
LUC ACTIVITY
+
+
−
−
NUCLEAR EXTRACT
IRRELEVANT
COMPLEMENTARY
IRRELEVANT
COMPLEMENTARY
OLIGONUCLEOTIDE

FIG. 103.

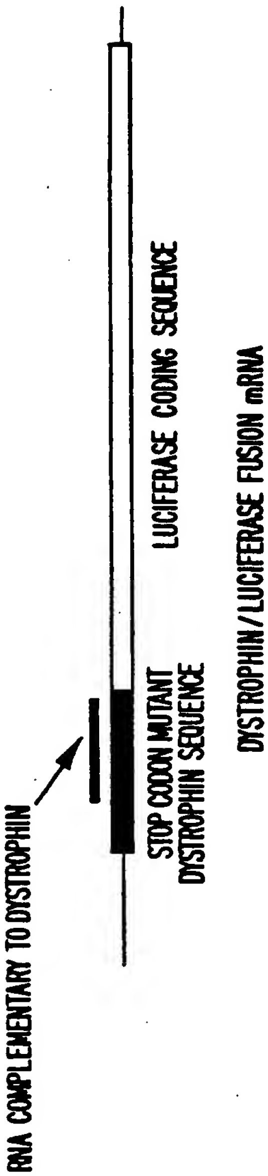
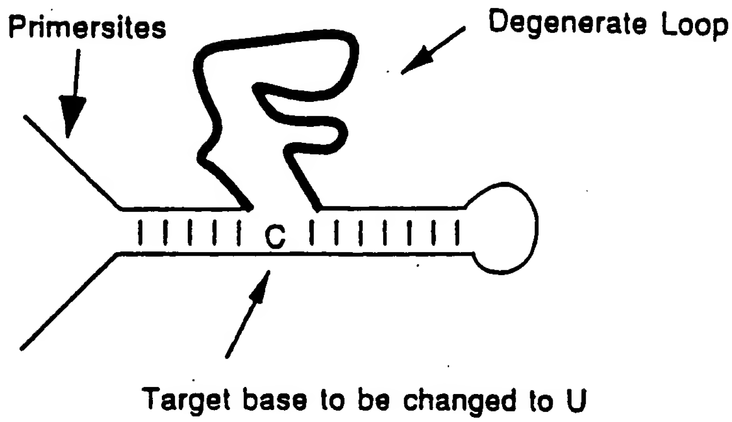
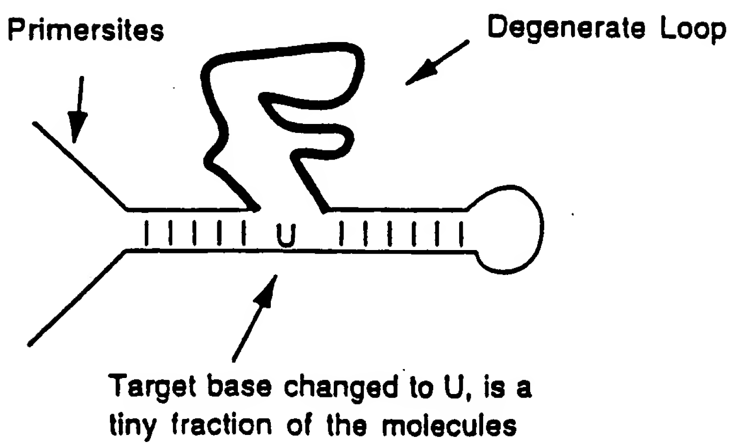

LUCIFERASE CODING SEQUENCE
STOP CODON MUTANT
DYSTROPHIN SEQUENCE
DYSTROPHIN/LUCIFERASE FUSION mRNA
RNA COMPLEMENTARY TO DYSTROPHIN

FIG. 104.

FIG. 105.



Incubate
+/- Cofactor



Convert to DNA, Select for mol cul s with the C to T bas chang . And rep at cycl s

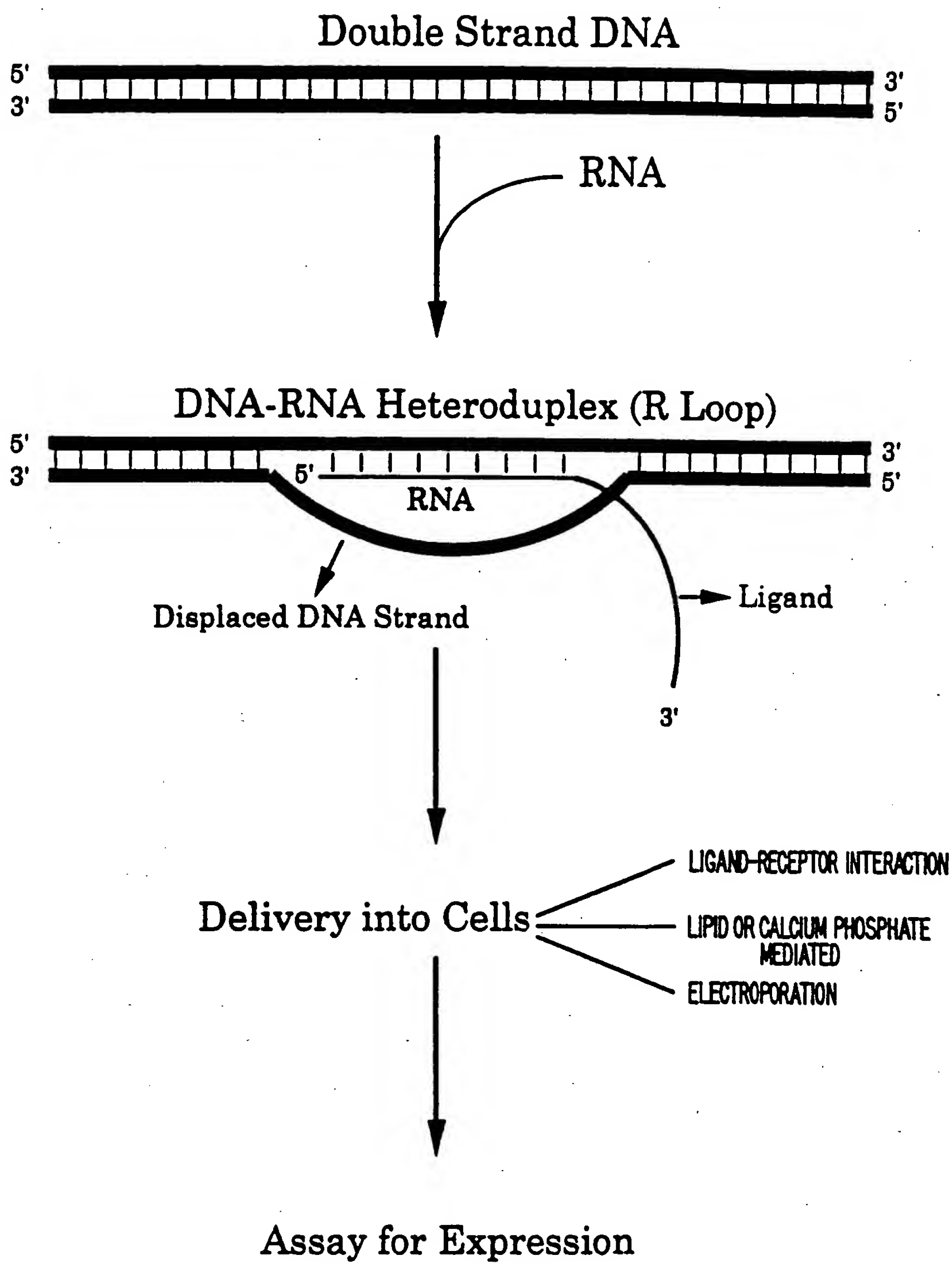

Double Strand DNA
5'
3'
3'
5'
RNA
DNA-RNA Heteroduplex (R Loop)
5'
3'
5'
RNA
3'
5'
Displaced DNA Strand
Ligand
3'
Delivery into Cells
LIGAND-RECEPTOR INTERACTION
LIPID OR CALCIUM PHOSPHATE MEDIATED
ELECTROPORATION
Assay for Expression

FIG. 106.

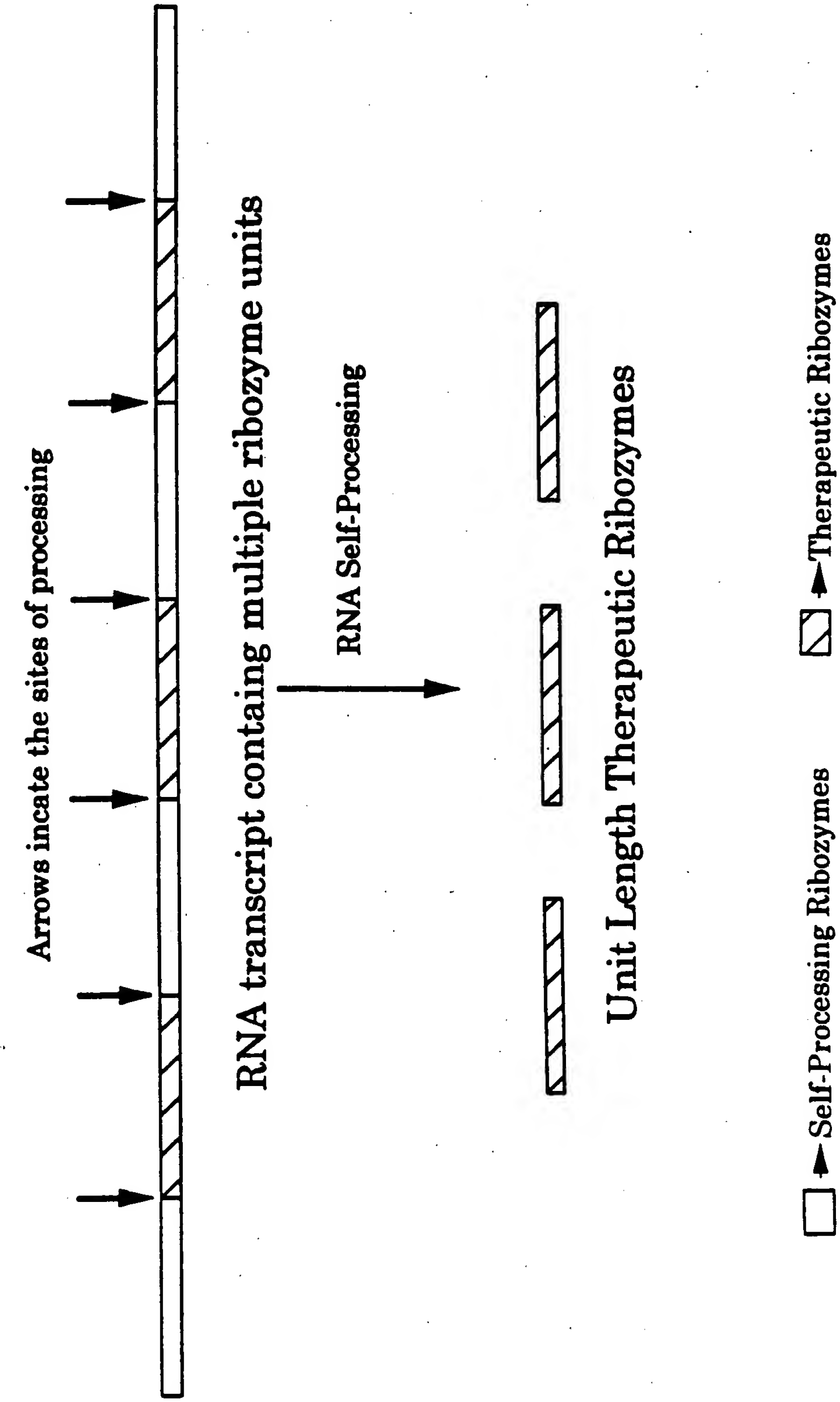

Arrows incate the sites of processing
RNA transcript containg multiple ribozyme units
RNA Self-Processing
Unit Length Therapeutic Ribozymes
Self-Processing Ribozymes
Therapeutic Ribozymes

FIG. 107.

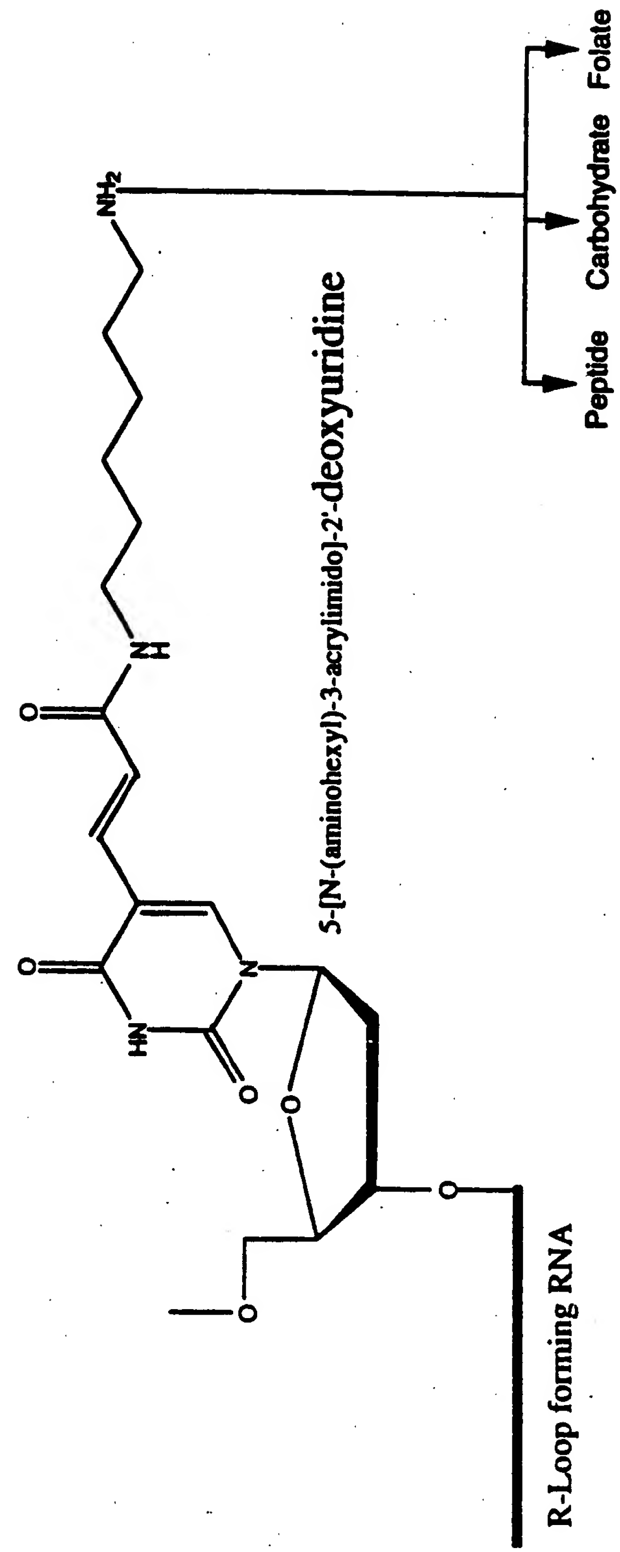

5-[N-(aminohexyl)-3-acrylimido]-2'-deoxyuridine
Peptide
Carbohydrate
Folate
R-Loop forming RNA

FIG. 108.